Figure 1: Examples of Nuclease Stable Ribozyme Motifs

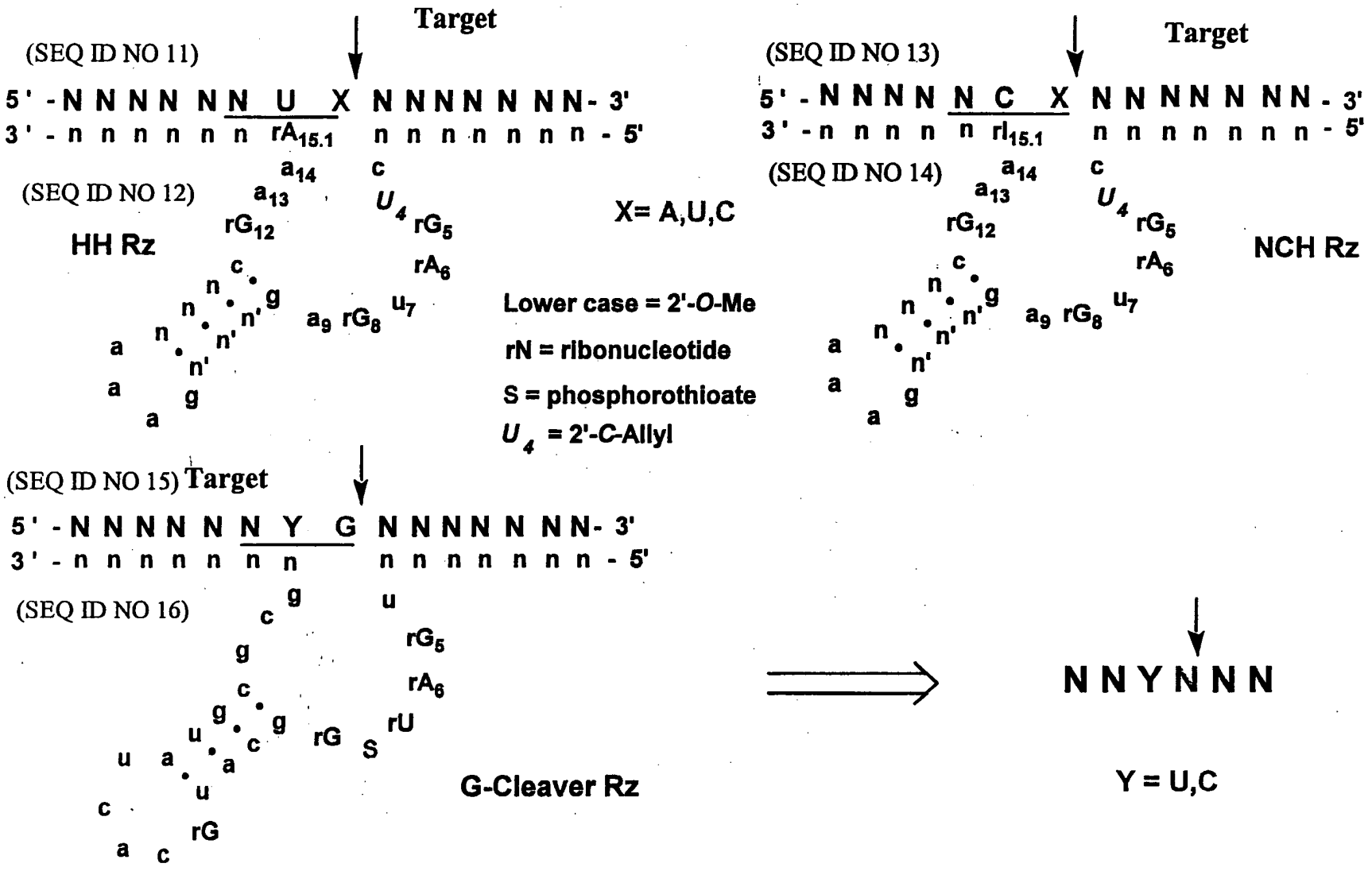


Figure 2: 2'-*O*-Me substituted Amberzyme Enzymatic Nucleic Acid Motif

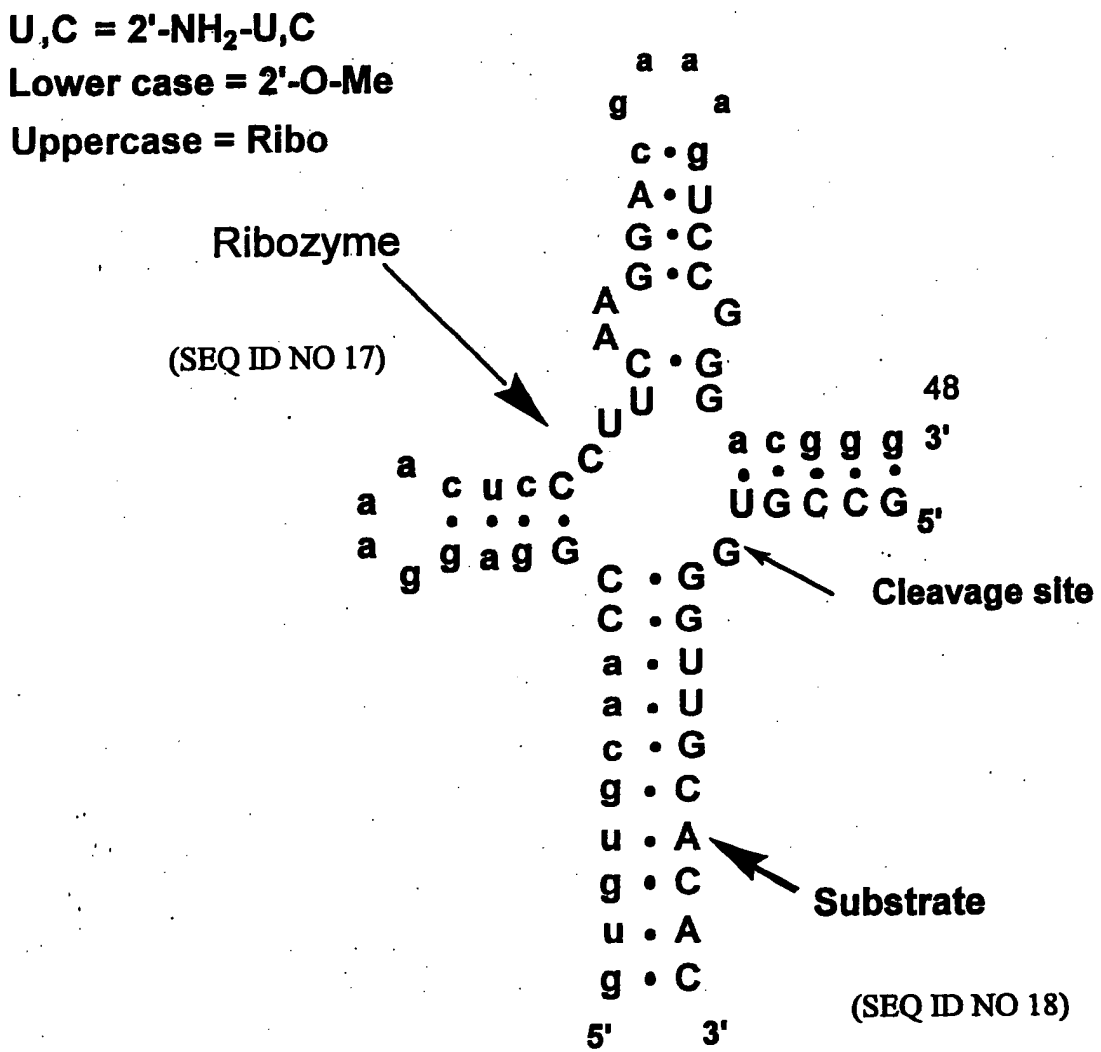
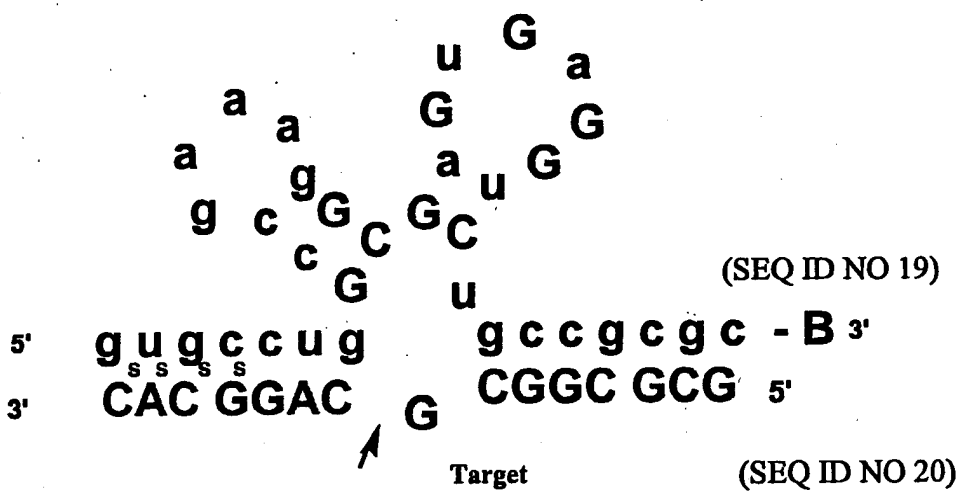


Figure 3: Stabilized Zinzyme Ribozyme Motif

Zinzyme A-motif RZ



(SEQ ID NO 19)

(SEQ ID NO 20)

Legend

Uppercase indicates natural ribo residues

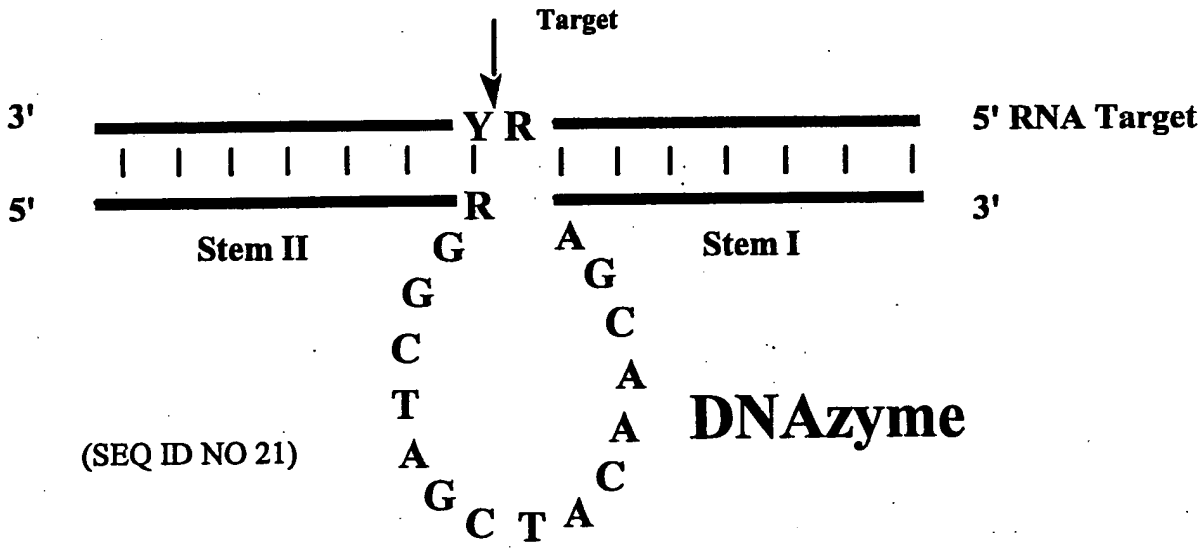
C indicates 2' - d-NH_2-C

Lowercase: 2'-*O*- Me

Subscript $_S$ indicates phosphothioate linkage

B: 3'-3' abasic moiety

Figure 4: DNAzyme Motif



Legend

Y = U or C
R = A or G

Figure 5. Detection of Target Sequence Using a Cis-Blocking Sequence

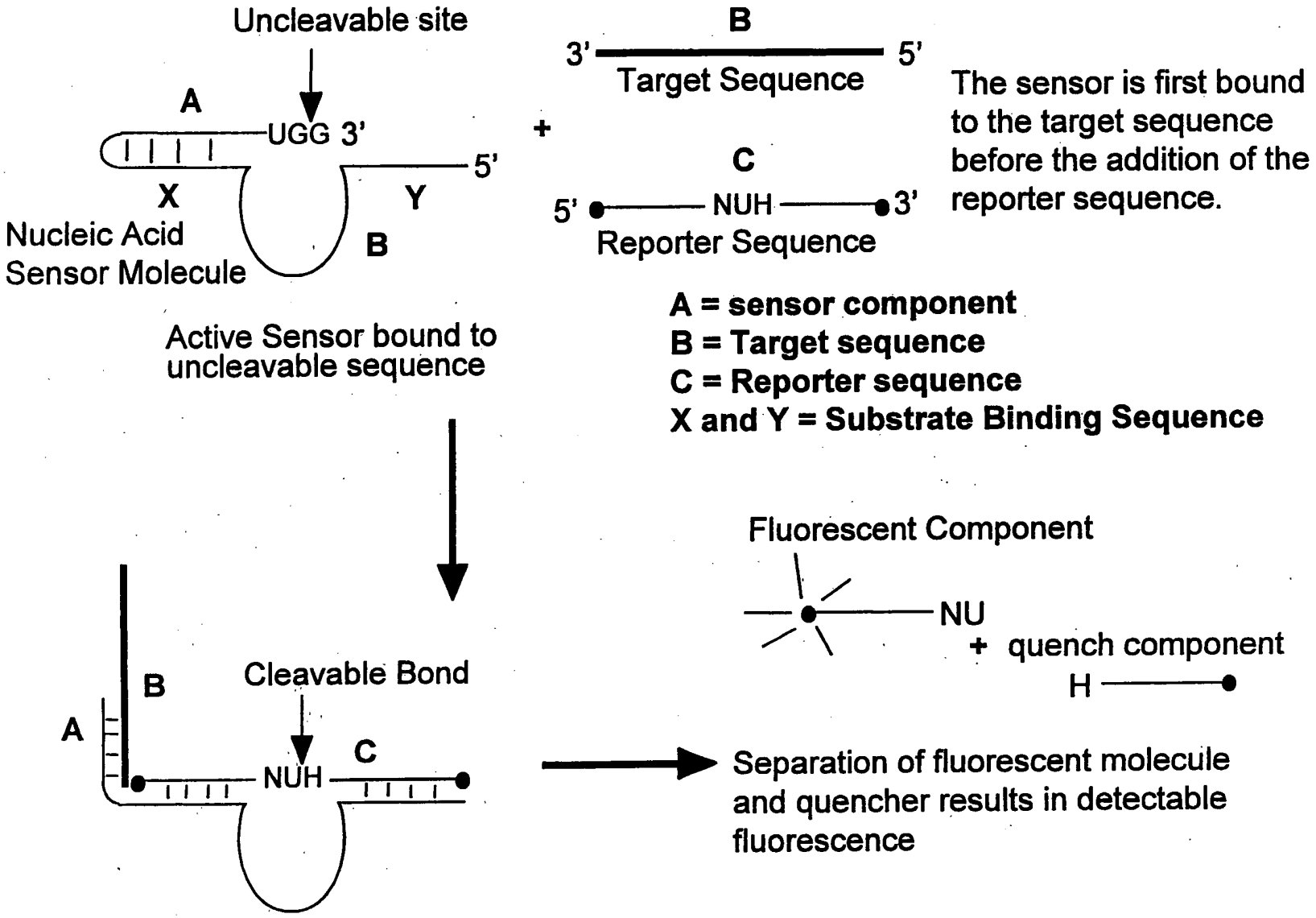


Figure 6. Schematic Diagram Representing the Two Primary Configurations of the Diagnostic effector molecule

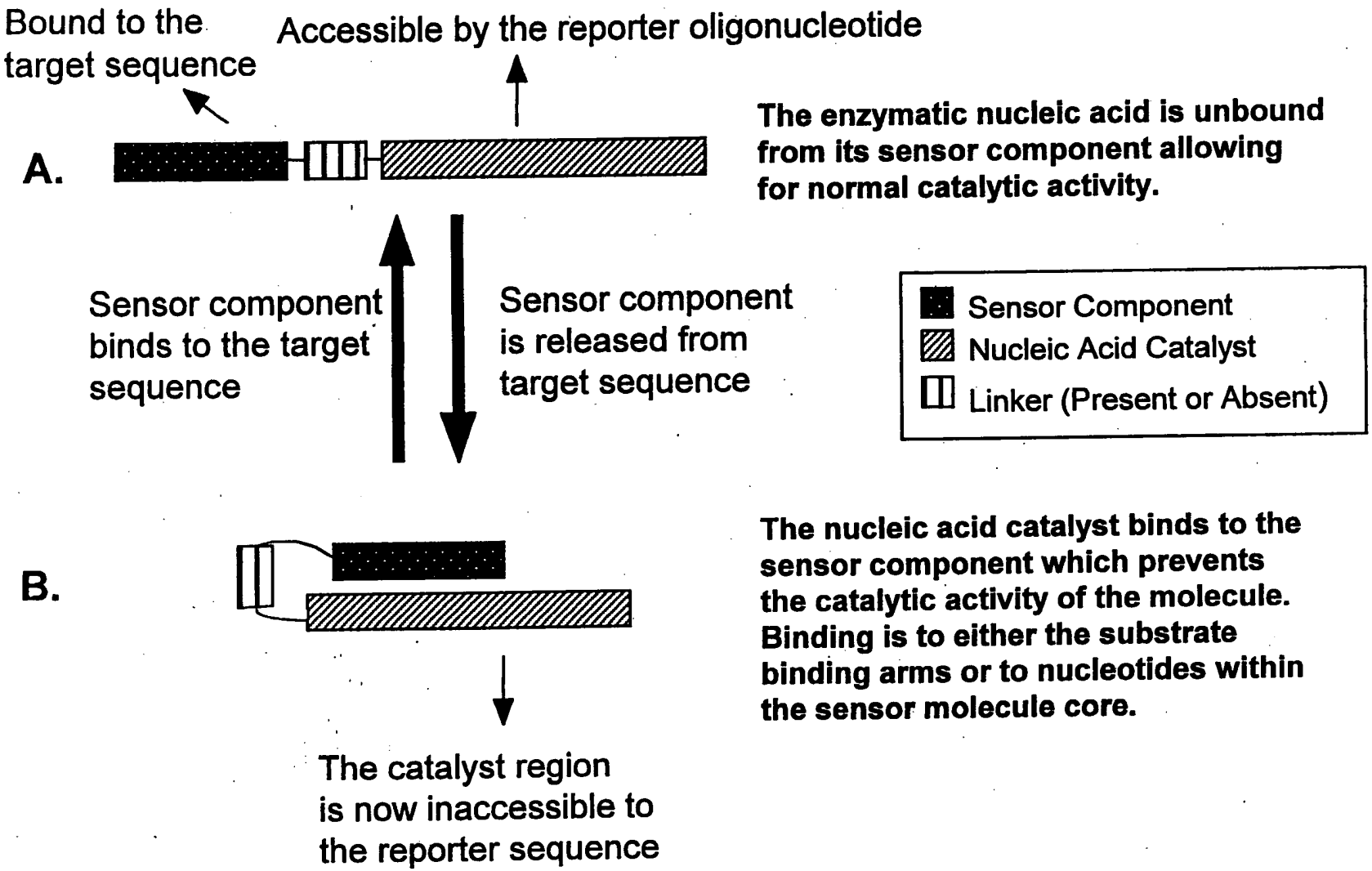


Figure 7. Examples of Nucleic Acid Sensor Molecules

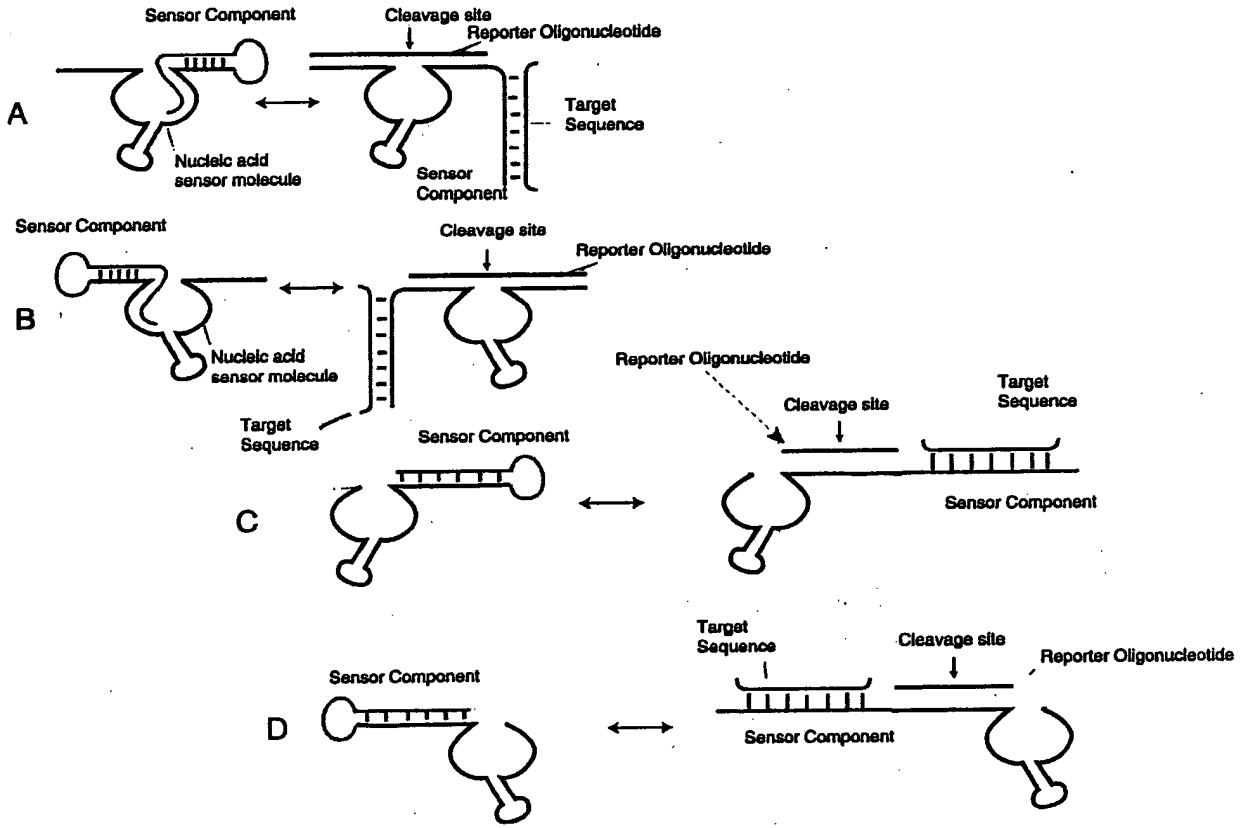


Figure 8. Examples of Nucleic Acid Sensor Molecules

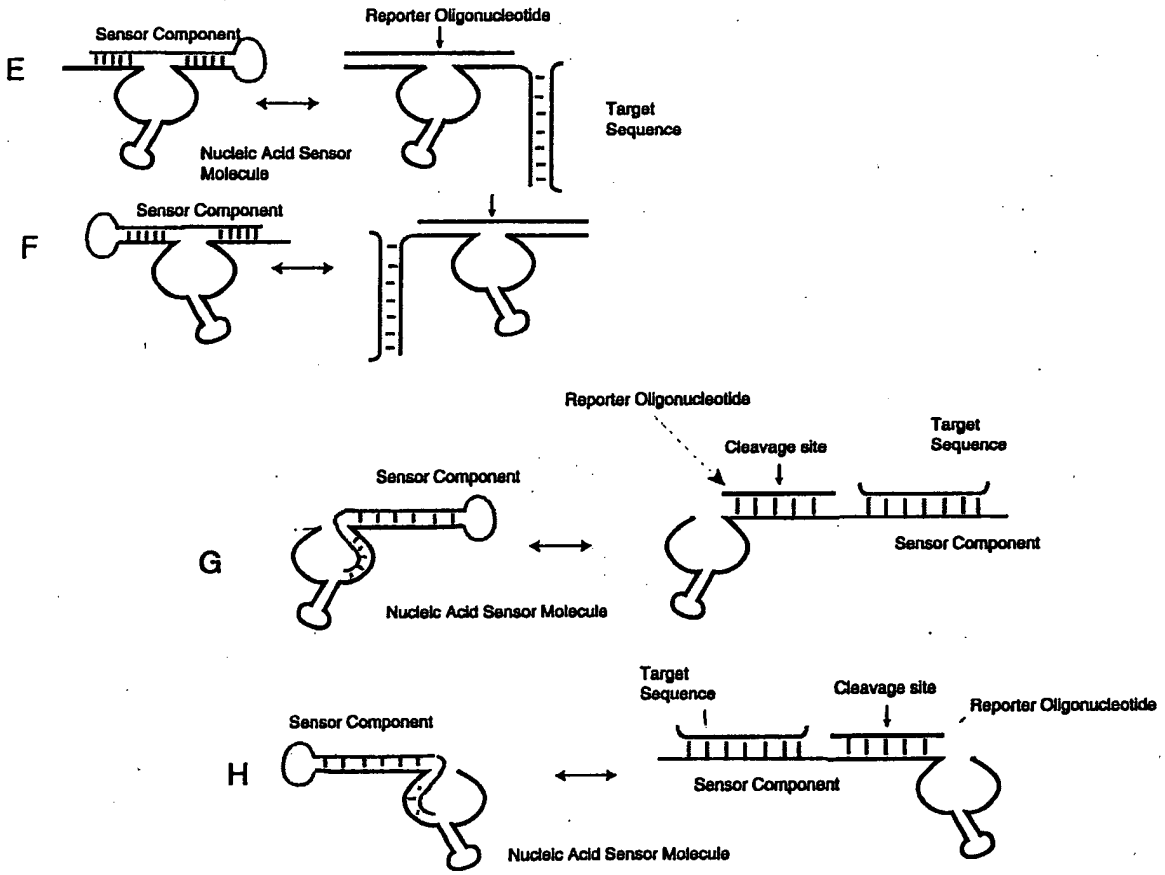


Figure 9. Examples of Nucleic Acid Sensor Molecules

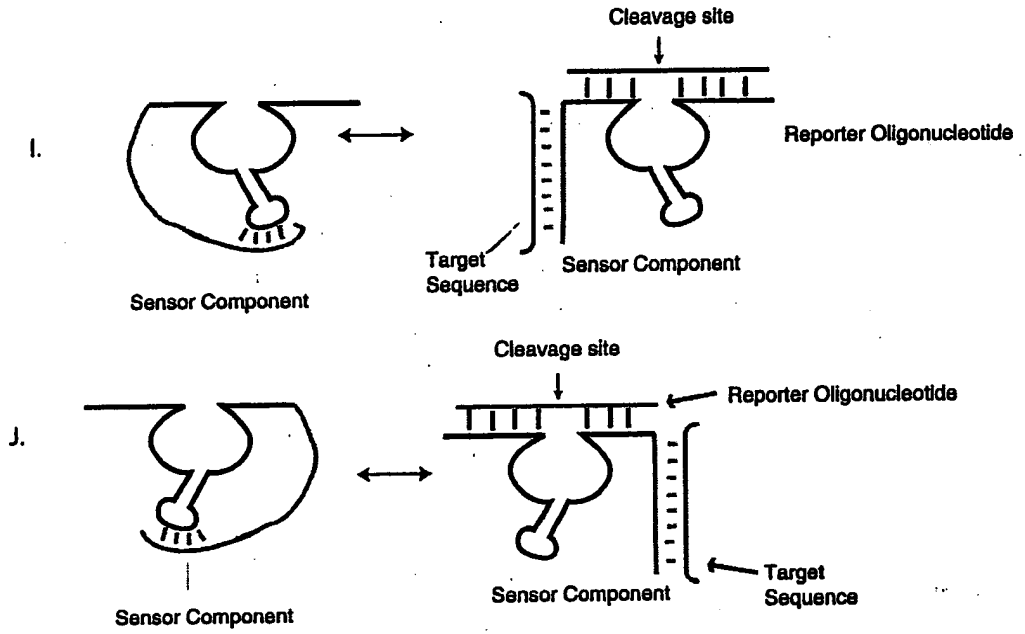
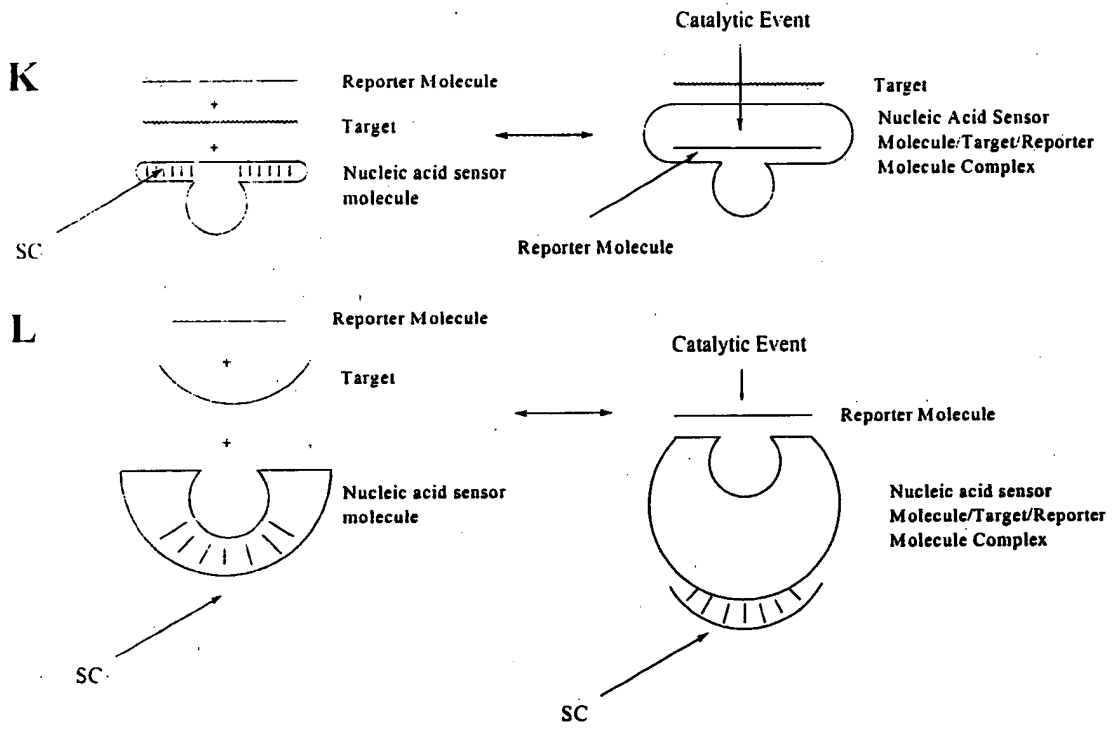


Figure 10: Examples of Nucleic Acid Sensor Molecules



SC = Sensor Component

Figure 11: Examples of Nucleic Acid Sensor Molecules

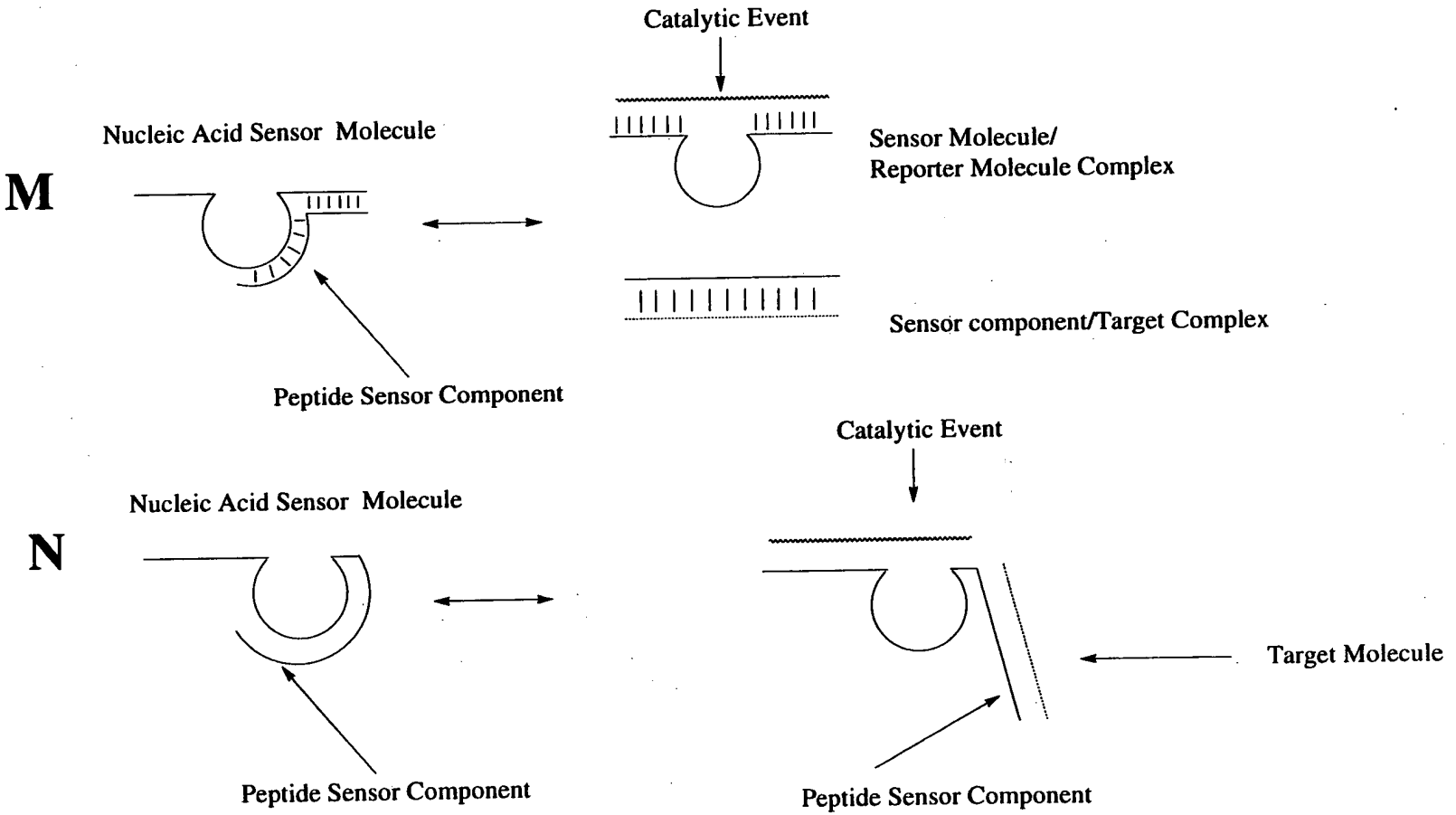
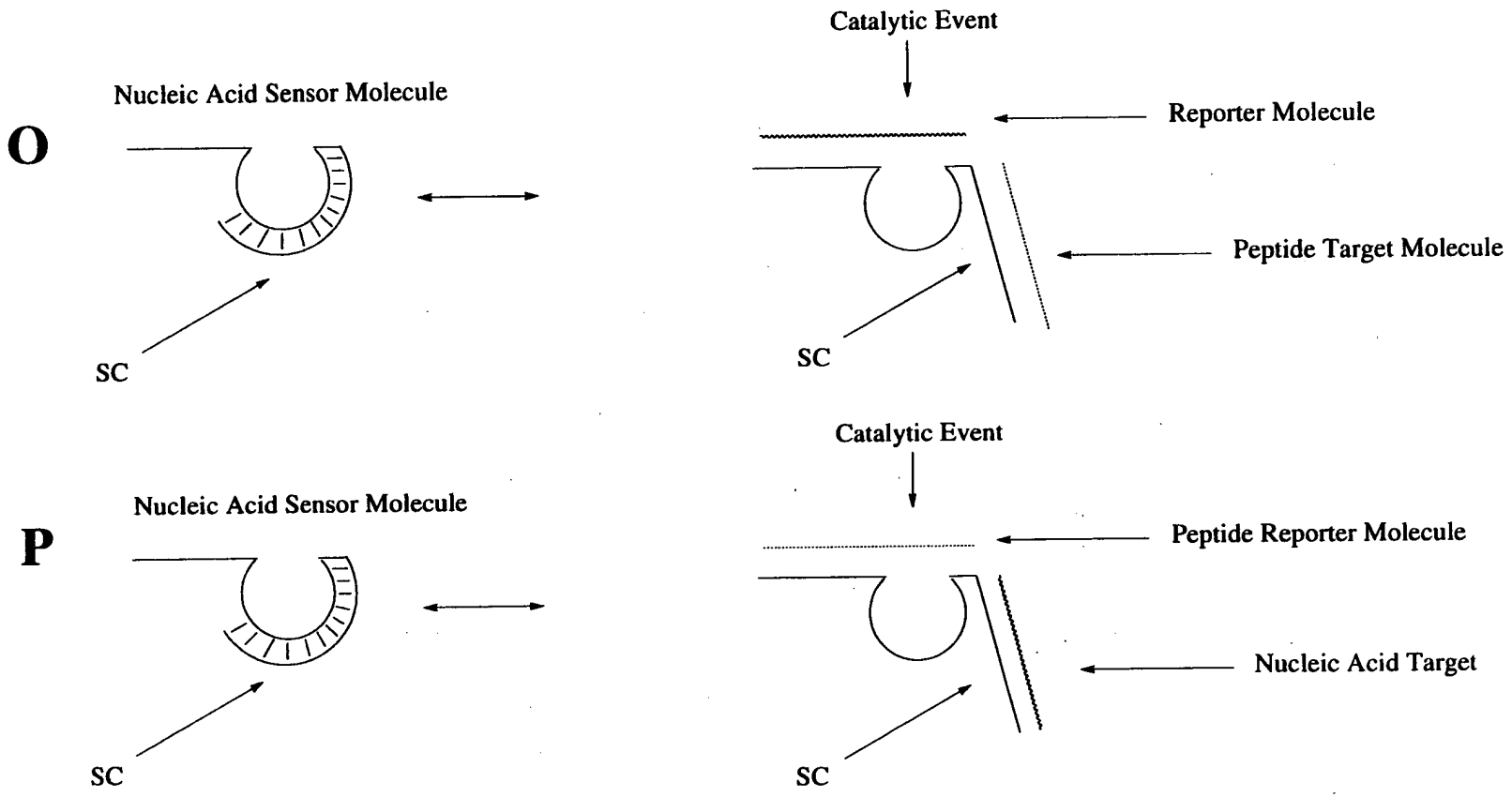


Figure 12: Examples of Nucleic Acid Sensor Molecules



SC = Sensor Component

Figure 13: Examples of Nucleic Acid Sensor Molecules

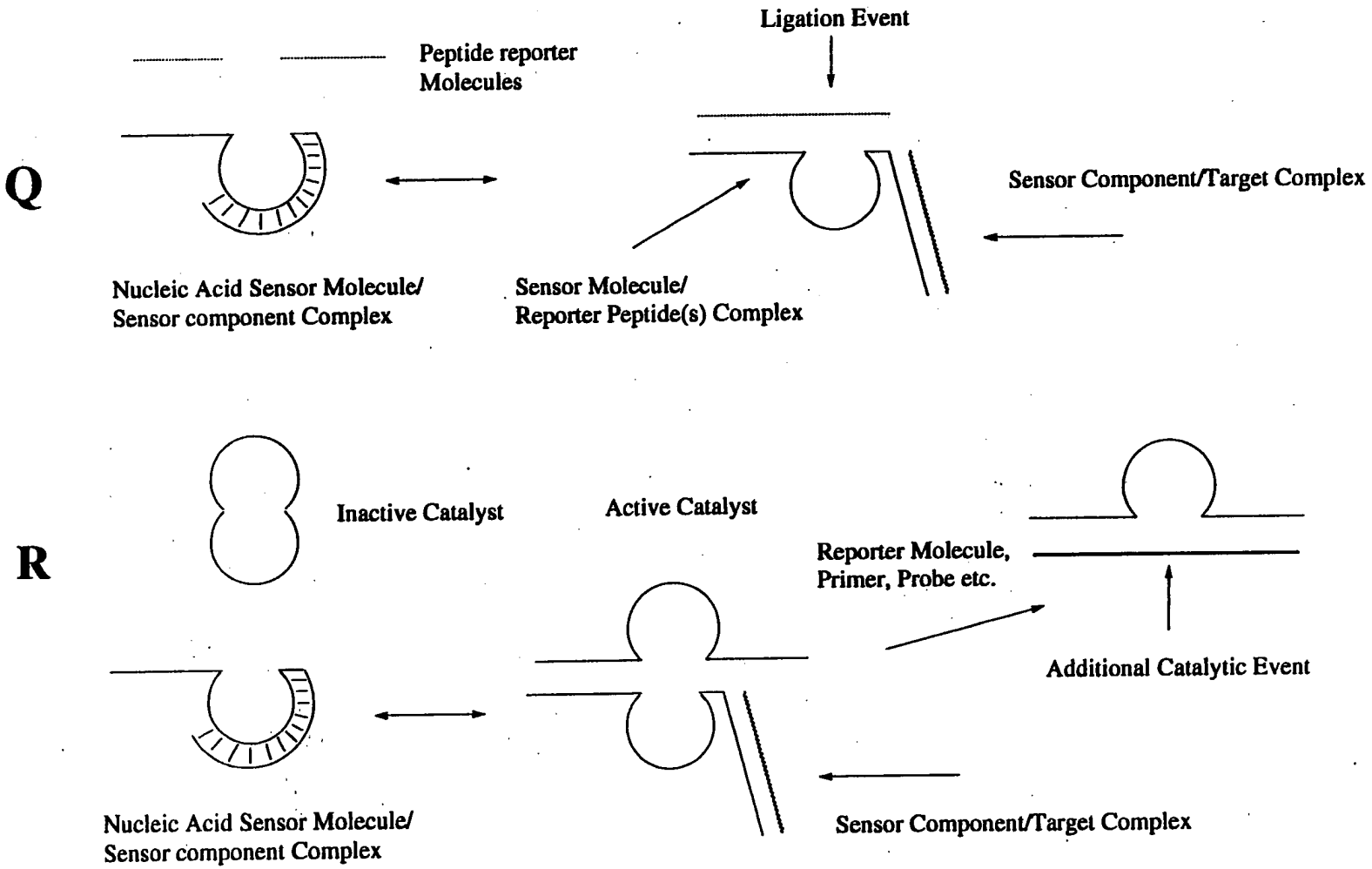


Figure 14: Inherent Amplification of Signal

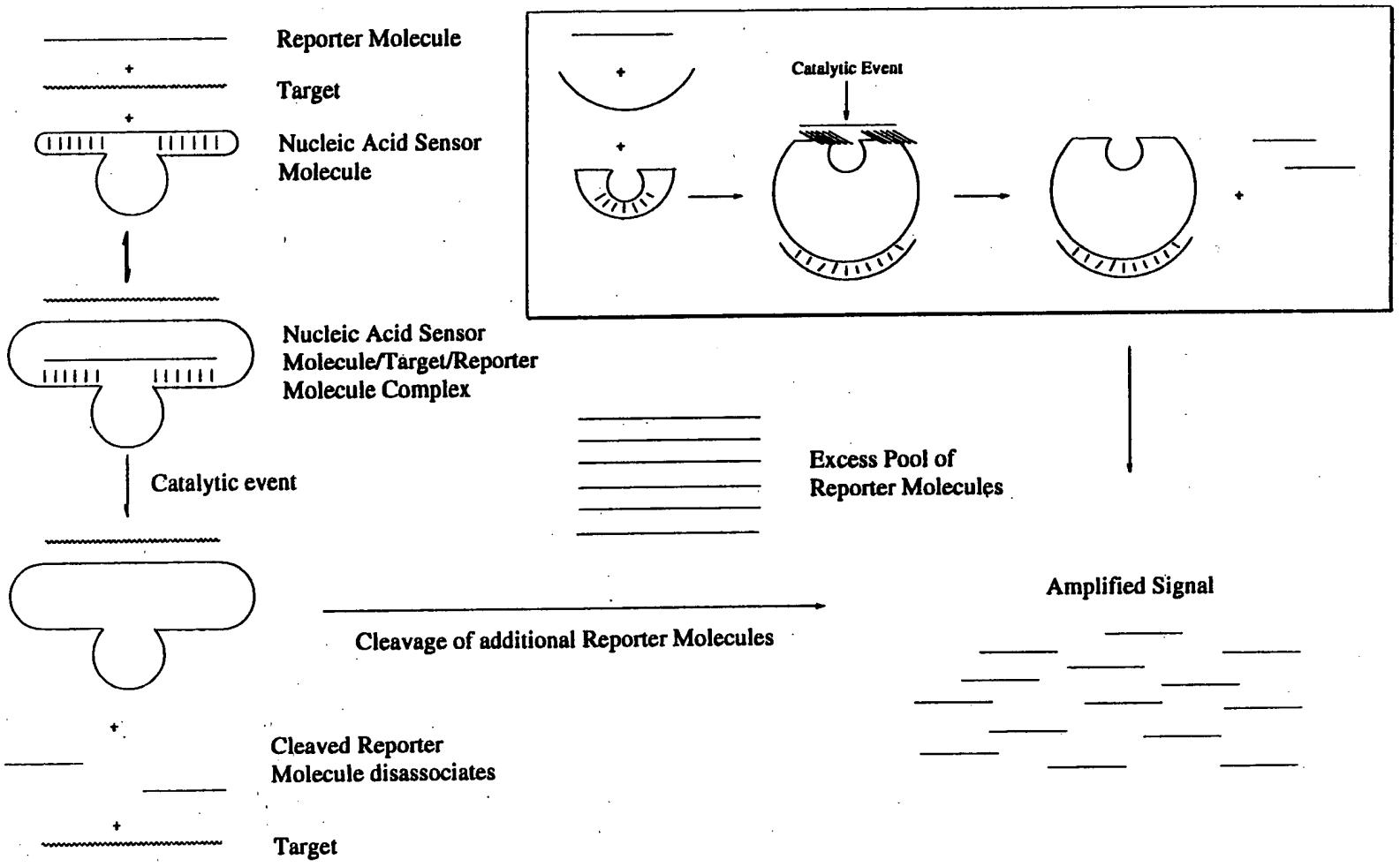


Figure 15: Example of Diagnostic System

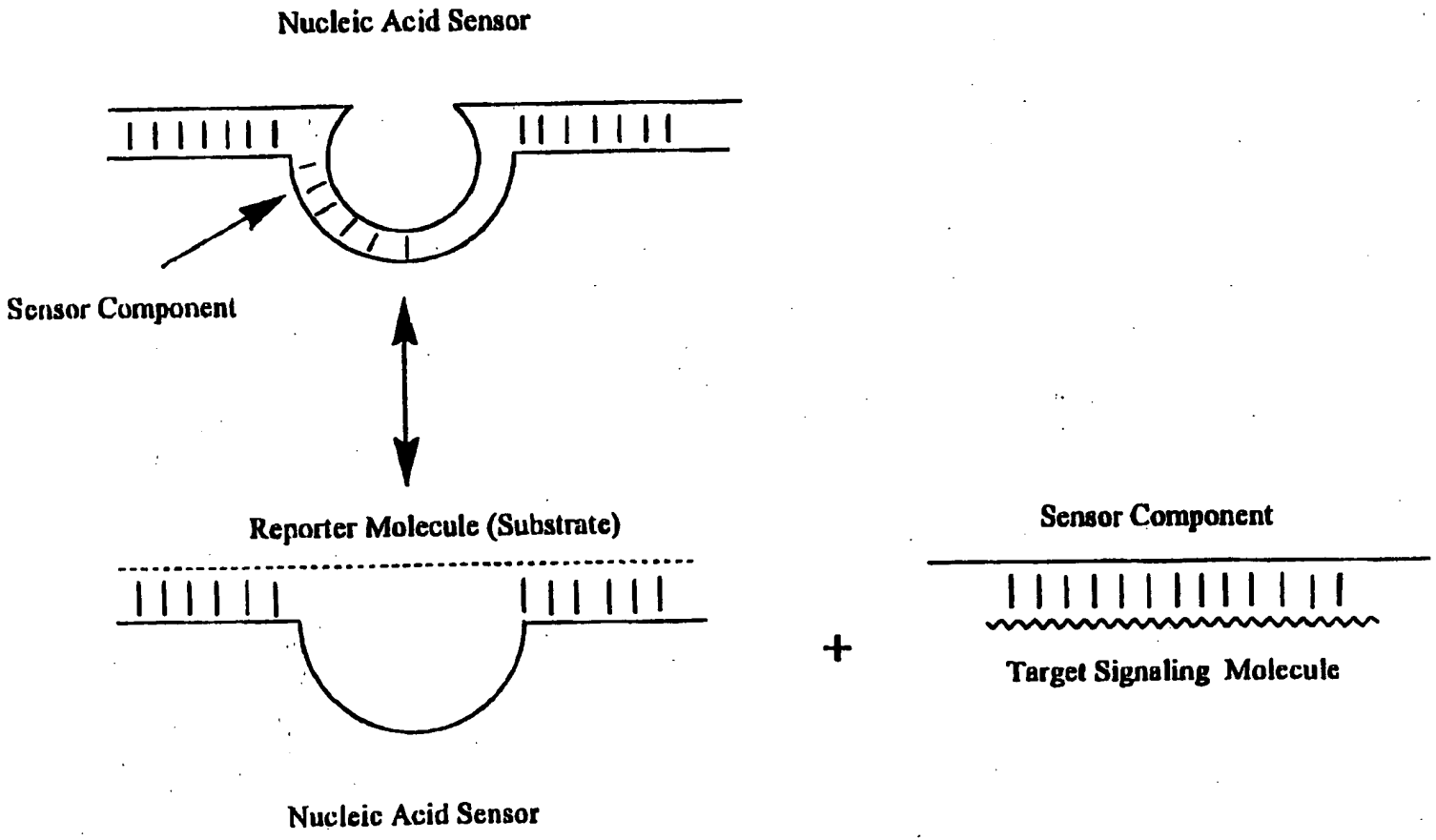


Figure 16: Ribozyme Diagnostic Screen

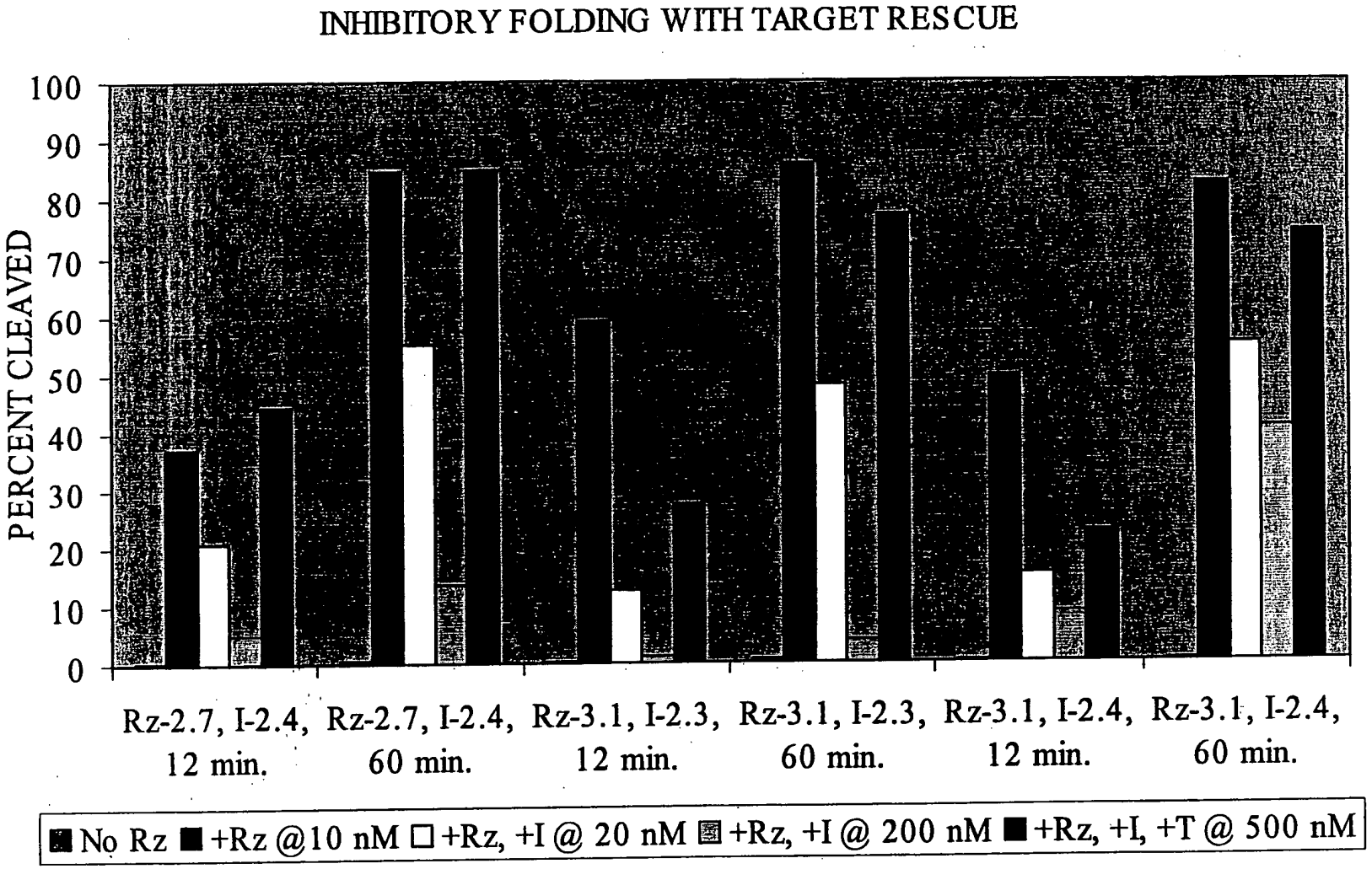


Figure 17a: Auto-ligation Nucleic Acid Sensor Molecules - Selection Scheme

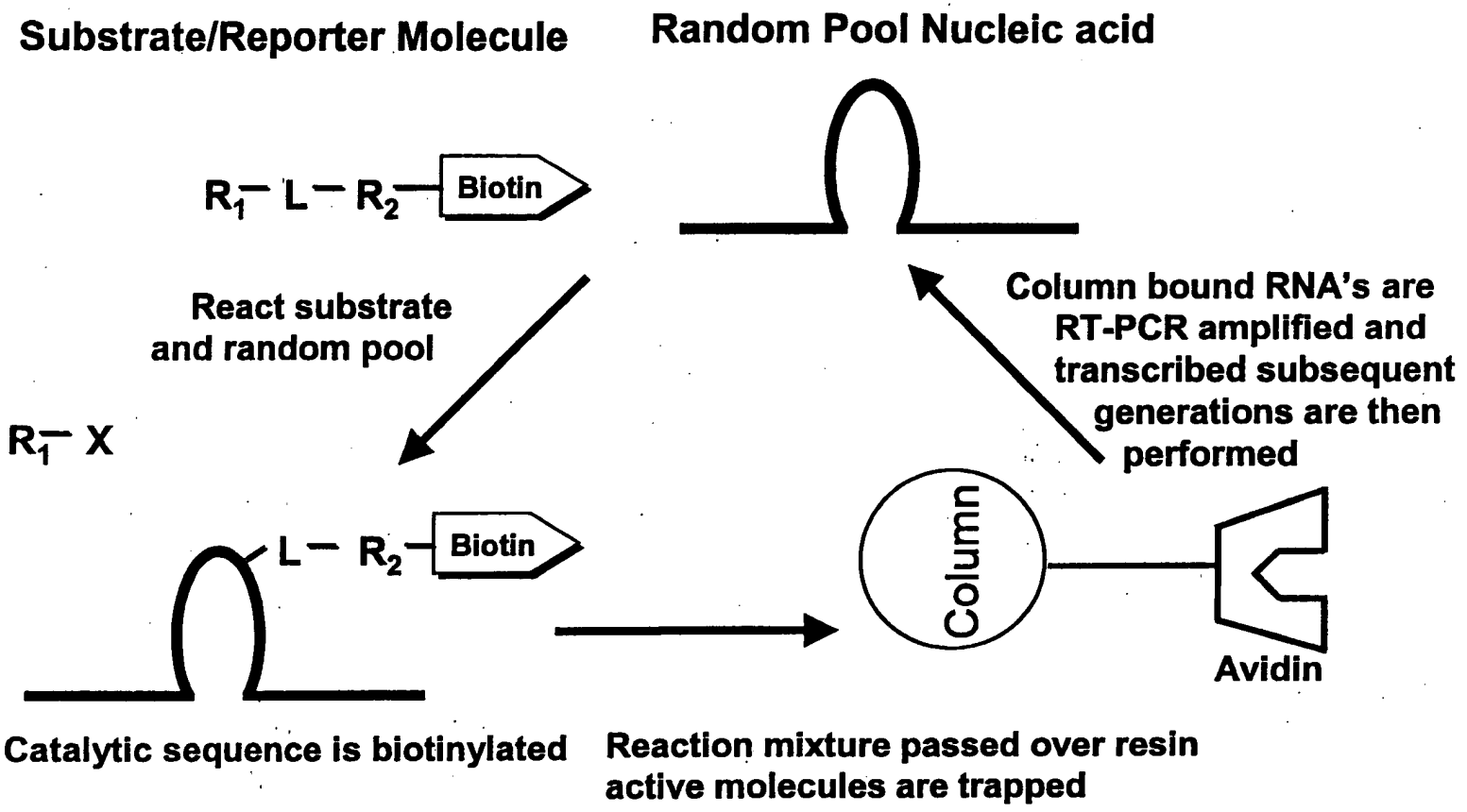
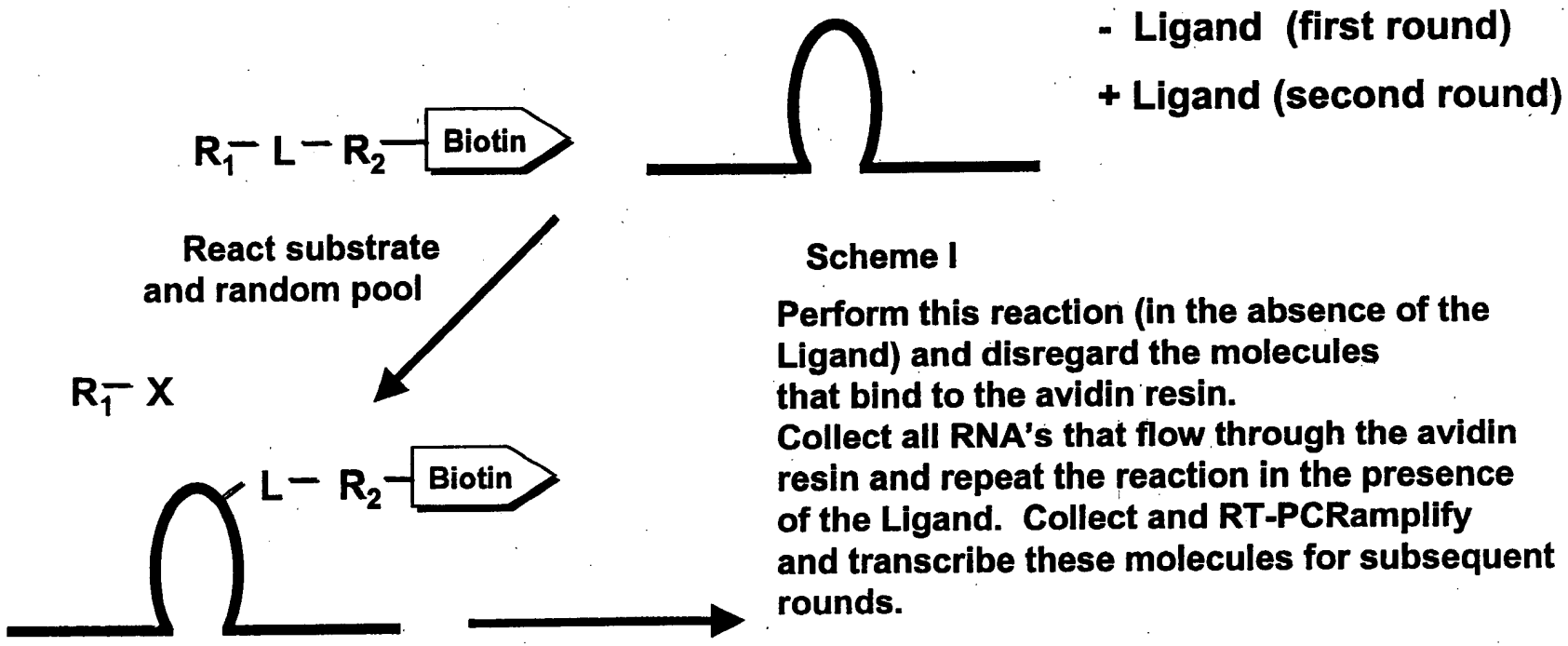


Figure 17b: Auto-ligation Nucleic Acid Sensor Molecules - Ligand Dependent

Substrate/Reporter Molecule + Random Pool Nucleic acid



- Ligand (first round)

+ Ligand (second round)

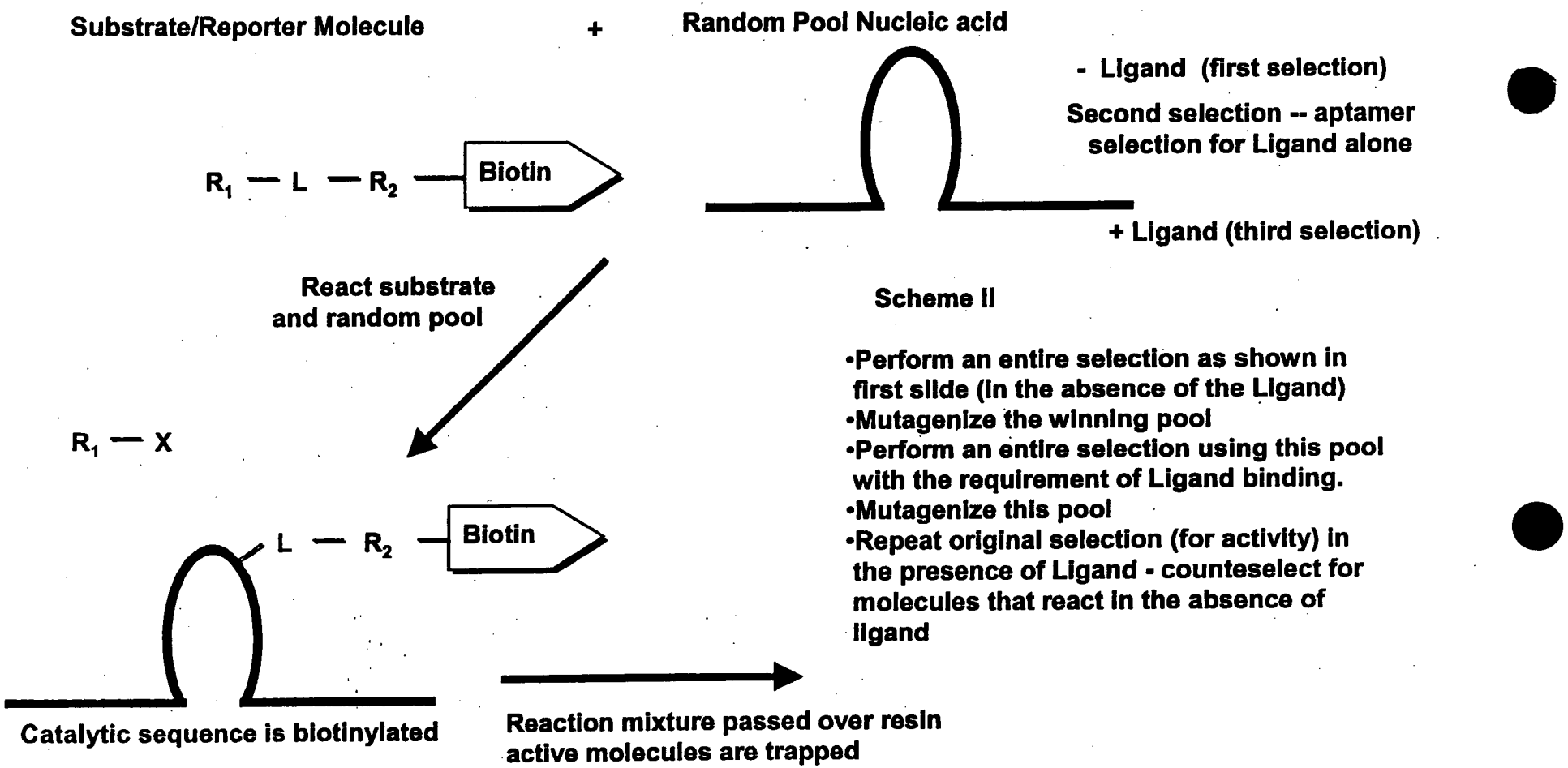
Scheme I

Perform this reaction (in the absence of the Ligand) and disregard the molecules that bind to the avidin resin. Collect all RNA's that flow through the avidin resin and repeat the reaction in the presence of the Ligand. Collect and RT-PCRamplify and transcribe these molecules for subsequent rounds.

Catalytic sequence is biotinylated

Reaction mixture passed over resin active molecules are trapped

Figure 17c: Auto-ligation Nucleic Acid Sensor Molecules - Ligand dependent



Scheme II

- Perform an entire selection as shown in first slide (in the absence of the Ligand)
- Mutagenize the winning pool
- Perform an entire selection using this pool with the requirement of Ligand binding.
- Mutagenize this pool
- Repeat original selection (for activity) in the presence of Ligand - counteselect for molecules that react in the absence of ligand

Figure 18a: Isomerase Nucleic Acid Sensor Molecule – Selection Scheme

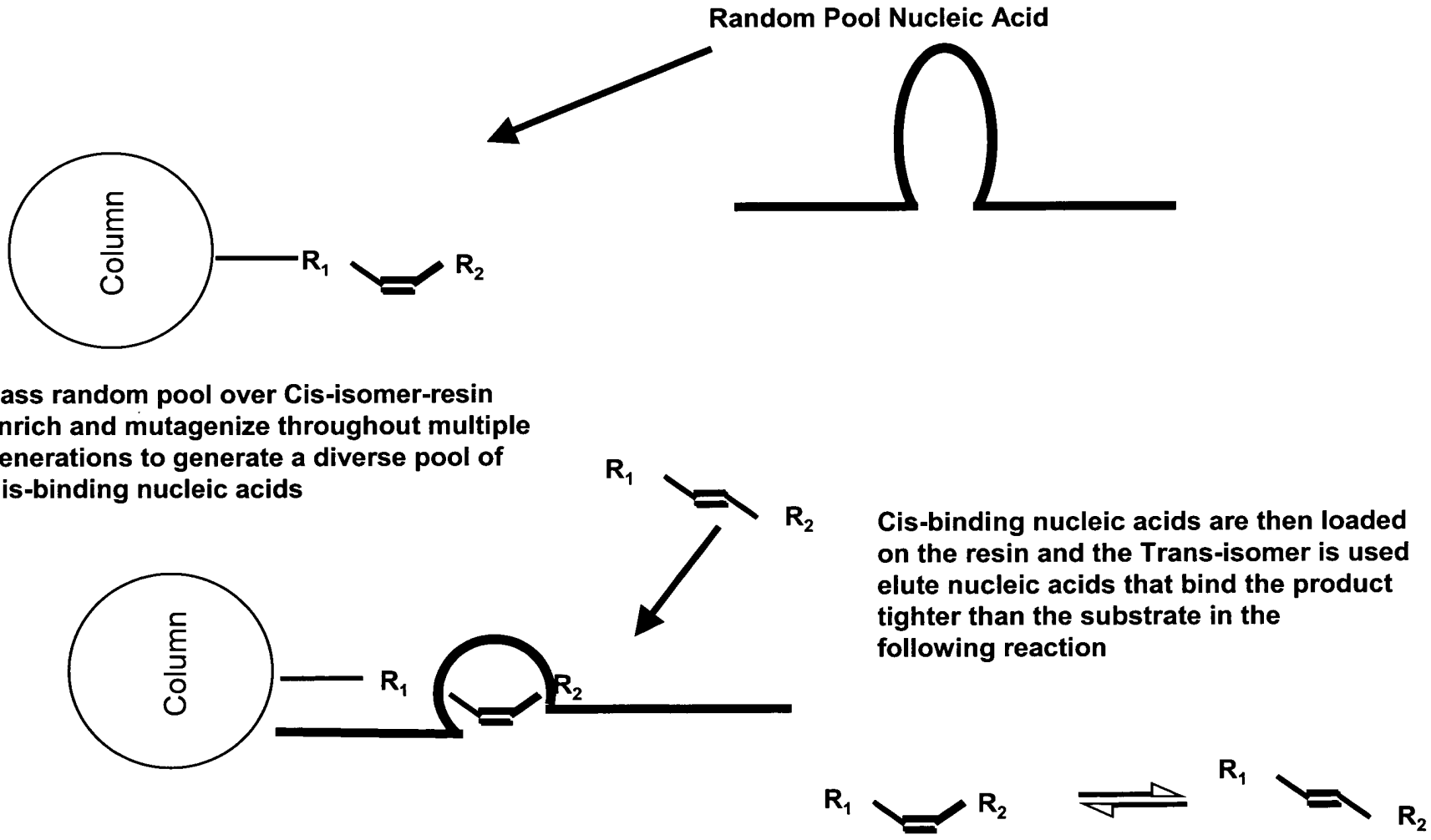
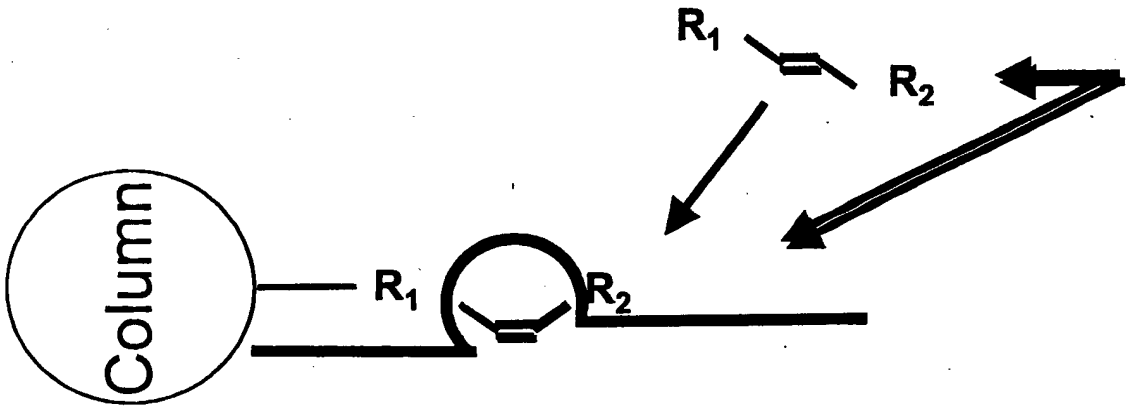


Figure 18b: Isomerase Nucleic Acid Sensor Molecule - Selection Scheme

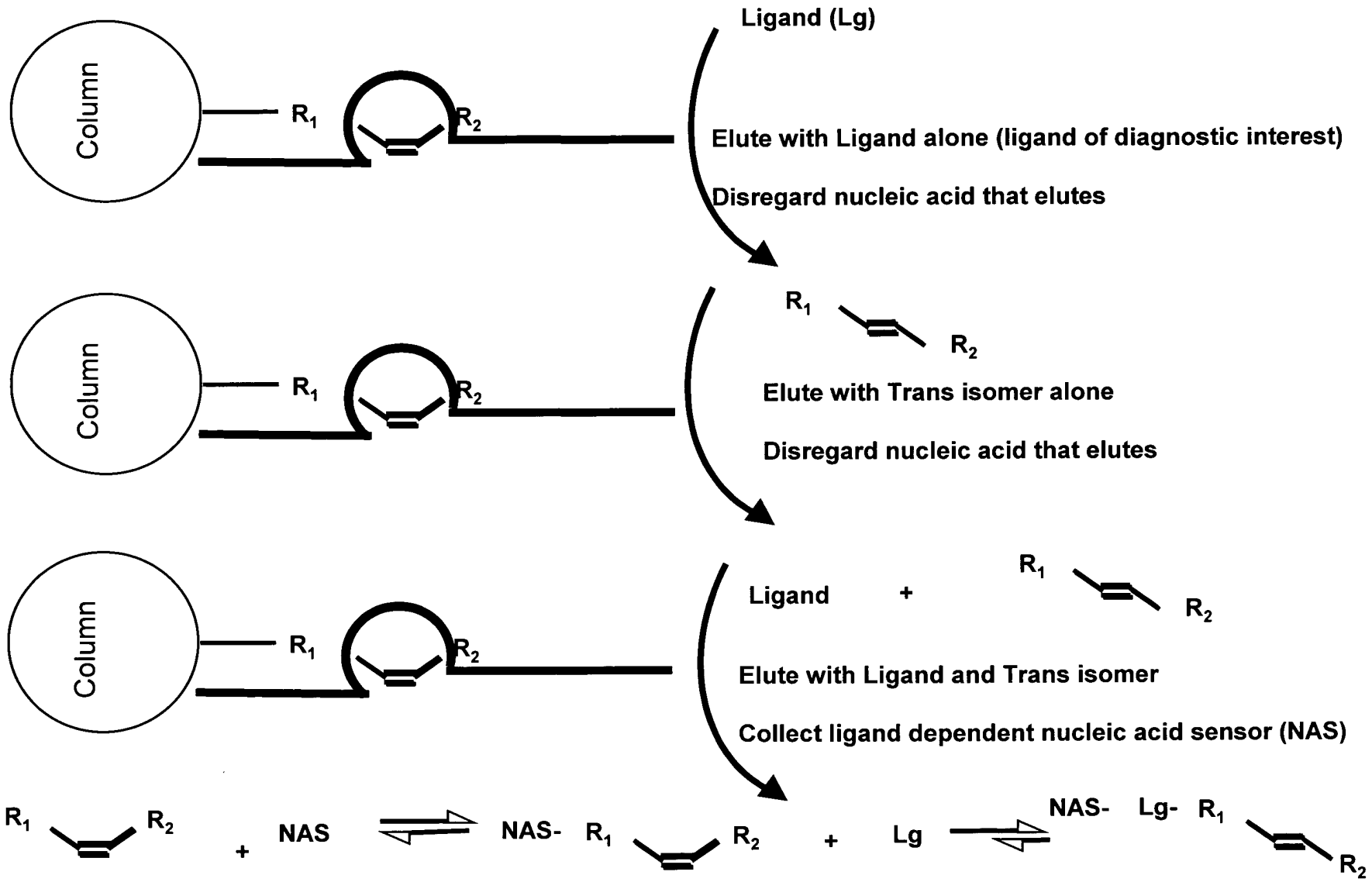


The concentration of ligand on the resin and the concentration of eluant ligand can be manipulated in order to bias for enzymatic nucleic acids that prefer one isomer over the other - and can therefore drive the reaction in a desired direction.

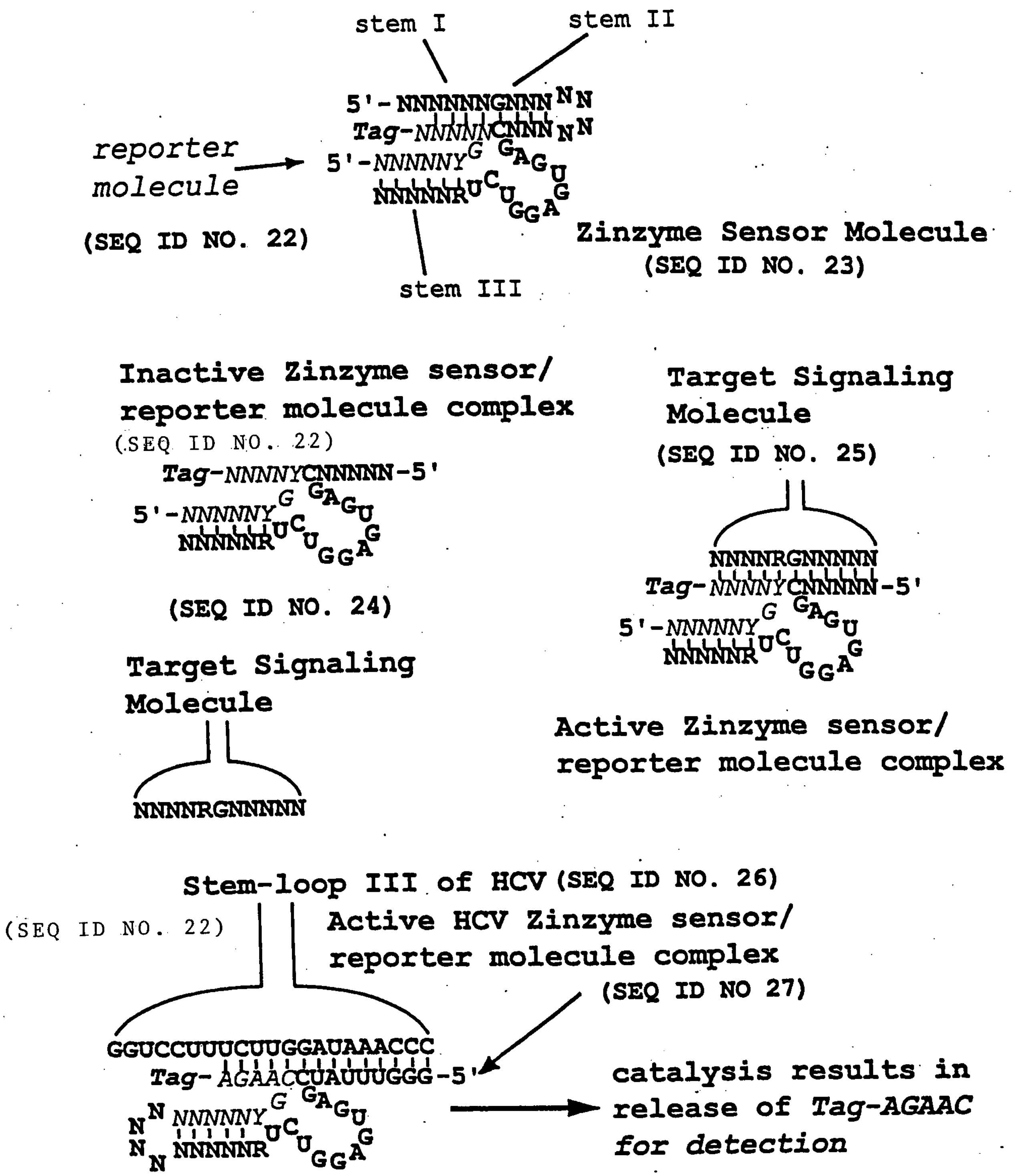
E.g. Selection for Cis-isomer at 100 uM - yeild $^{cis}K_d$ = 100 uM
Elute with Trans-isomer at 0.1 uM - yield $^{trans}K_d$ = 0.1 uM

Isolate catalysts for the reaction below

Figure 18c: Isomerase Nucleic Acid Sensor Molecule - Ligand dependent



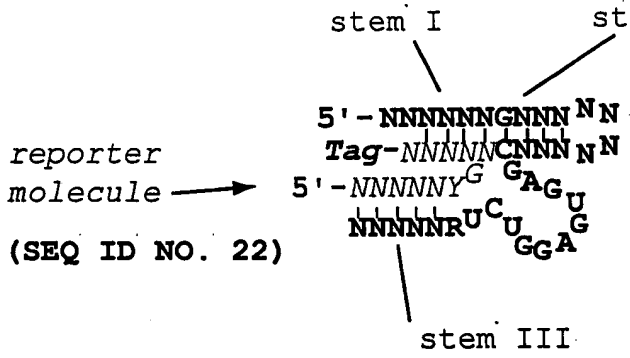
Zinzyme Sensor Molecule for detection of Nucleic Acid



Zinzyme sensor can be attached to solid support/surface, for example at the 5'-end

FIG. 19

Figure 20: Zinzyme Sensor Molecule for detection of Protein



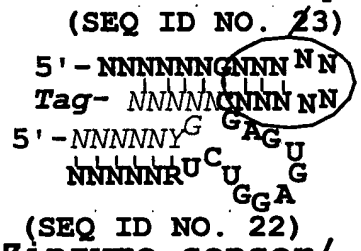
reporter molecule →

(SEQ ID NO. 22)

Zinzyme Sensor Molecule

(SEQ ID NO. 23)

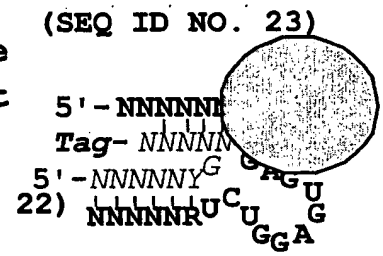
RNA sequence that binds a clinically relevant protein



Zinzyme sensor/ reporter molecule complex

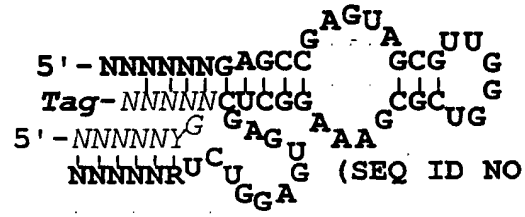
biological sample containing target protein

Protein interaction



Activated/Inactivated Complex

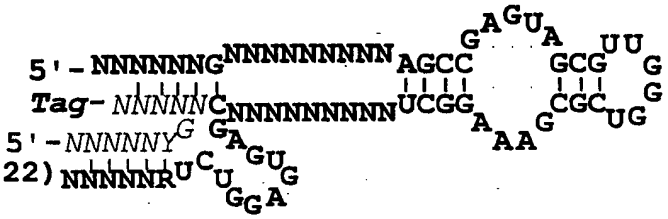
Sensor/reporter complex for detection of HCV core protein



(SEQ ID NO. 22)

HCV Zinzyme sensor with loop IIID of HCV (directs the binding of HCV core protein)

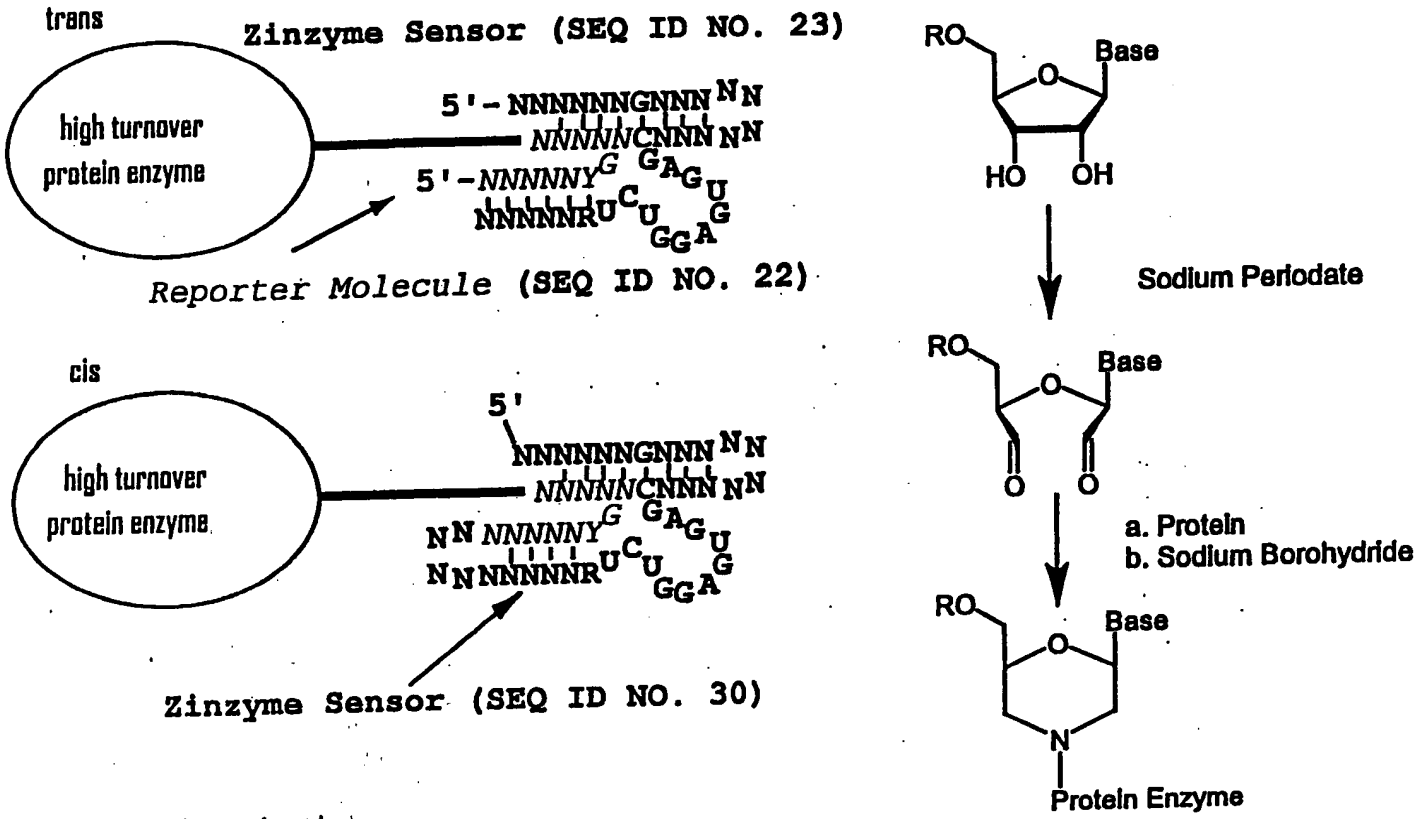
(SEQ ID NO 28)



HCV Zinzyme sensor with loop IIID of HCV connected via randomized linker

(SEQ ID NO 29)

Zinzyme Sensor Molecule with protein enzyme reporter

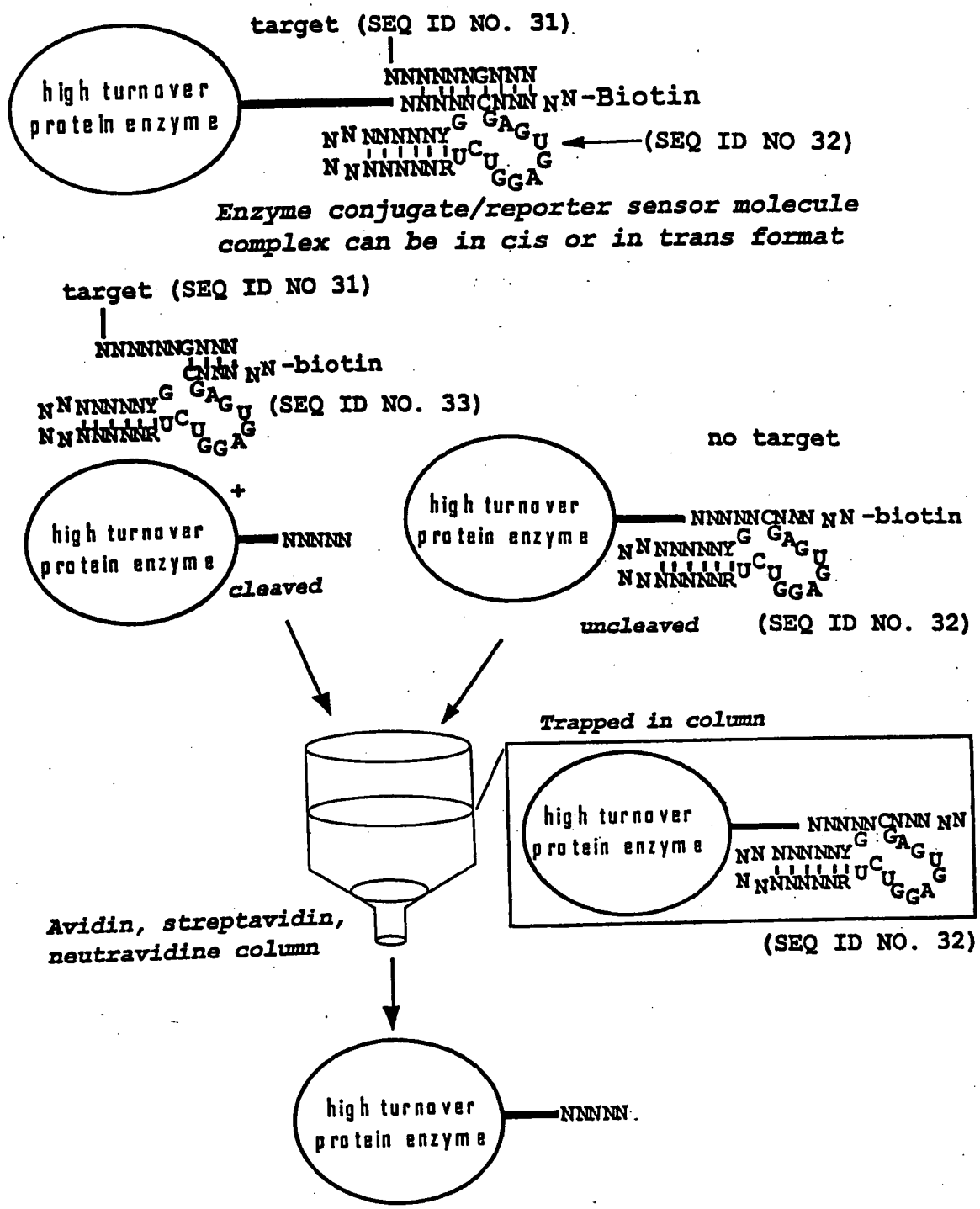


R is oligonucleotide.
Protein can be attached via amino linker.
Alternately, R is phorphoramidite moiety for incorporation at 5'-end of oligonucleotide.

High turnover protein enzyme is, for example, Luciferase, Horseradish peroxidase, beta-galactosidase, alkaline phosphatase.

FIG. 21

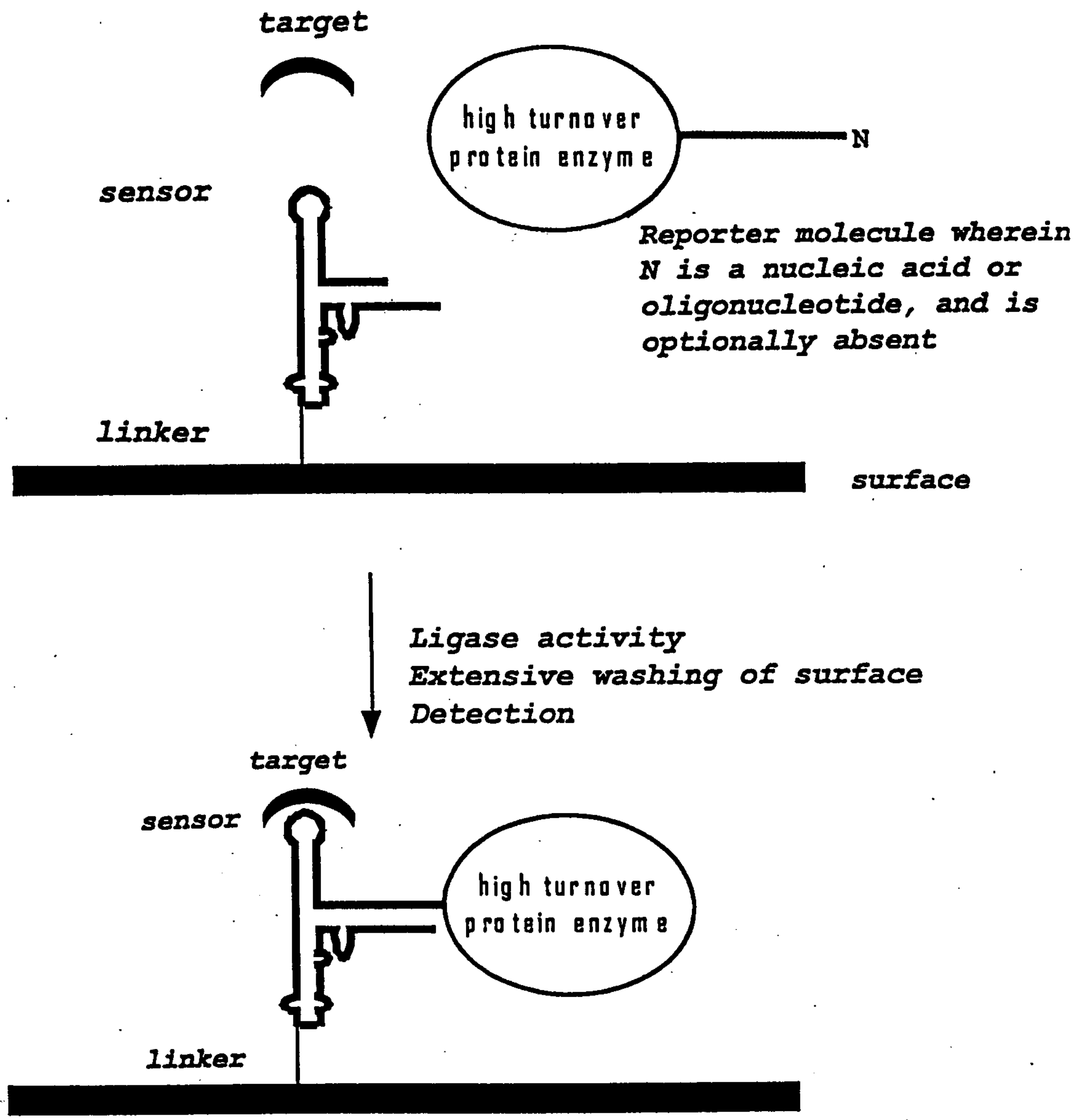
Amplification of signal via use of protein enzyme conjugate



Assay column eluent to determine enzyme activity proportional to sensor molecule activity.

FIG. 22

Ligase Sensor Molecule with enzymatic reporter



Alternatively, a fluorescent or chemiluminescent based reporter molecule is used.

FIG. 23

Figure 24: Selection of Nucleic Acid Sensor Molecules with Ligase Activity

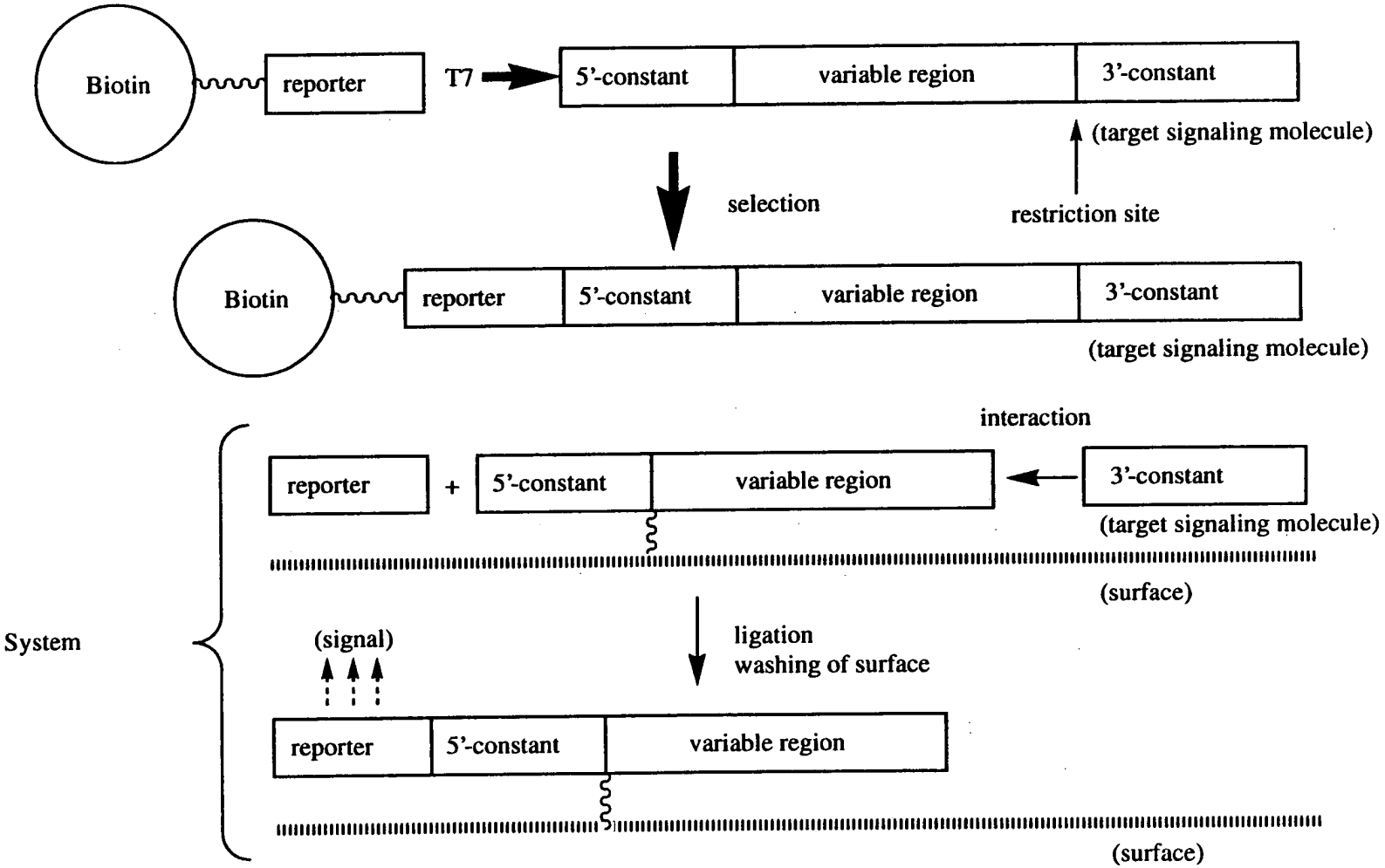
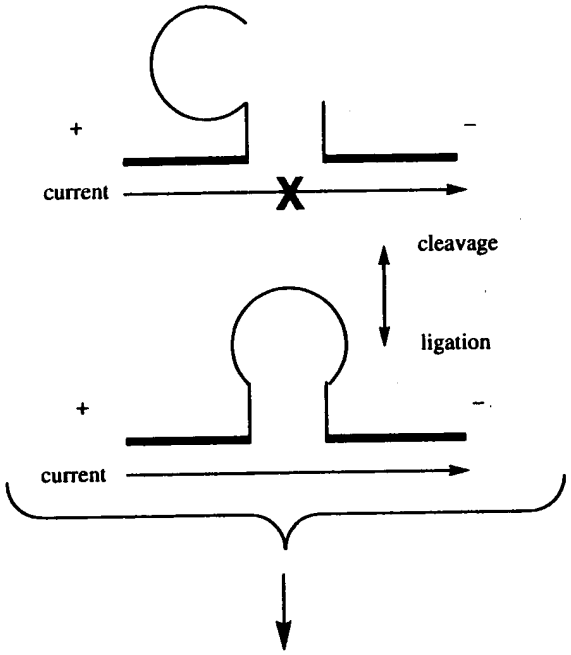


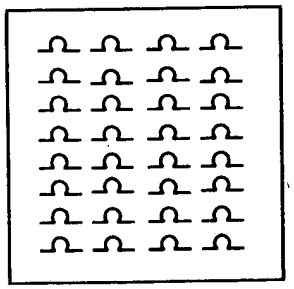
Figure 25: Nucleic Acid Sensor Molecule-Based Electric Cirucuit



Open Circuit

Closed Circuit

The Nucleic Acid Sensor Circuit responds to target signaling agents:
for example predeterminded levels of current, voltage, impedence, or target signaling molecules (nucleic acid sequences, peptides, neurotransmitters etc.) in a system.



Processor

N_x

DEVICE → **OUTPUT**

(computer)

Figure 26: Target Inactivation of Zinzyme Sensor Molecule

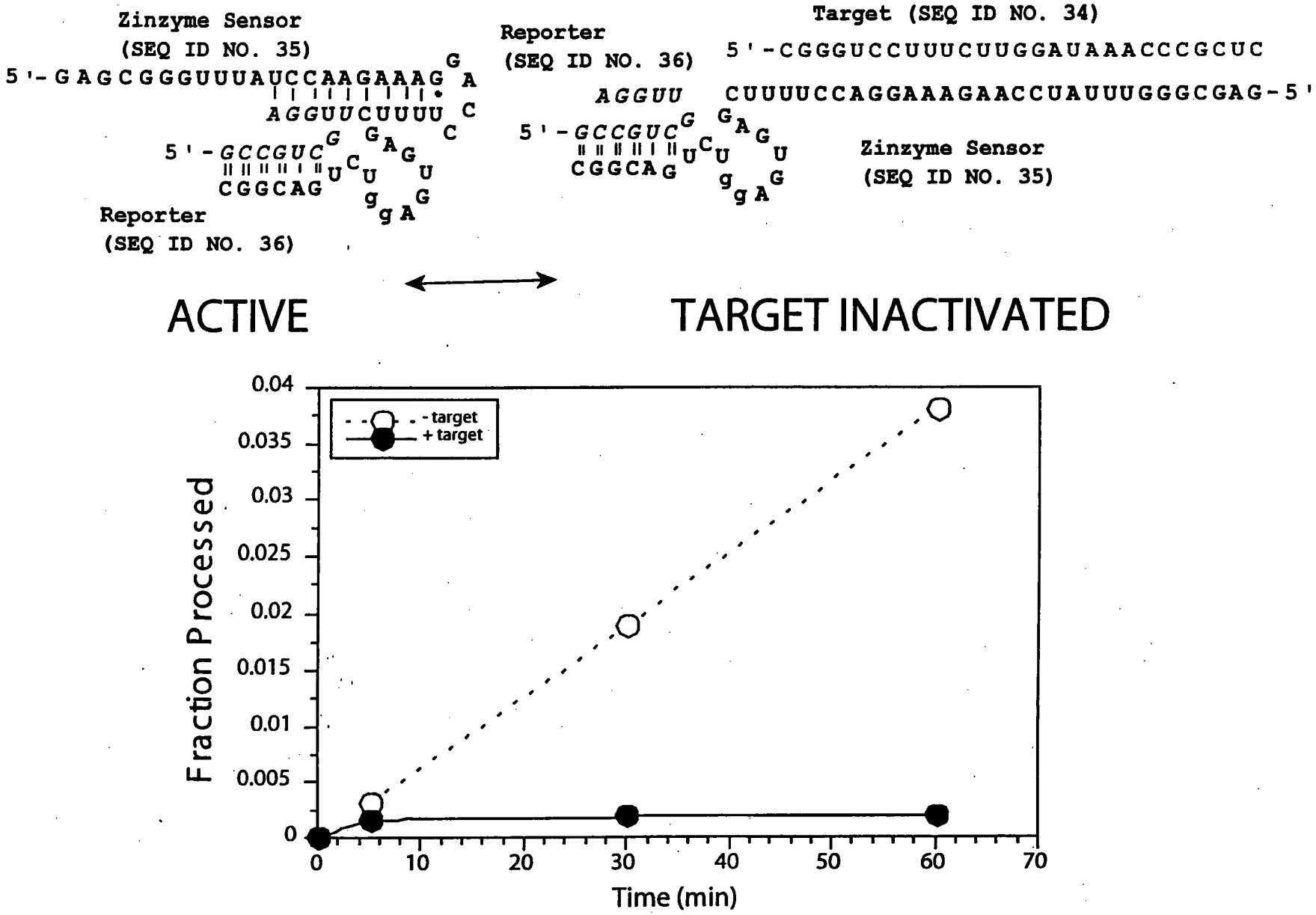


Figure 27: Target Activation of Zinzyme Sensor Molecule

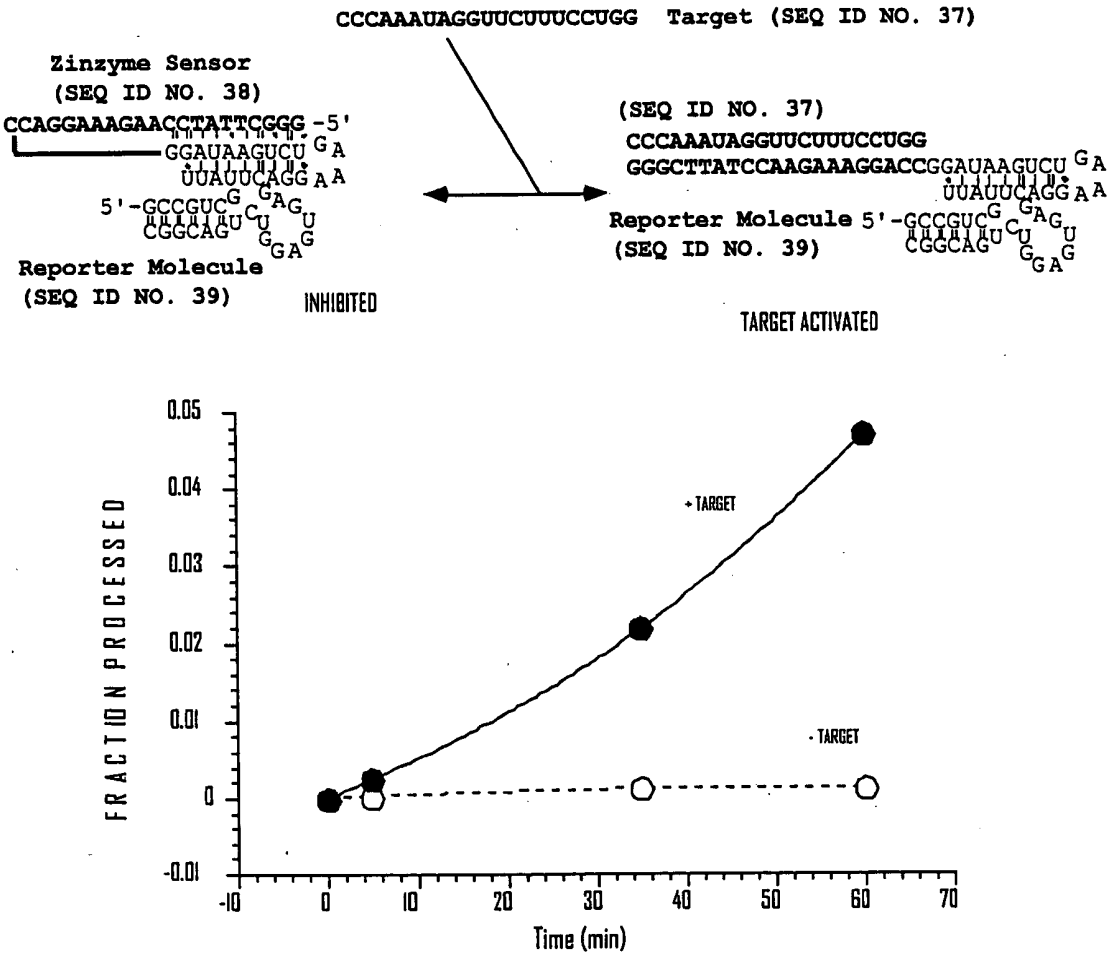
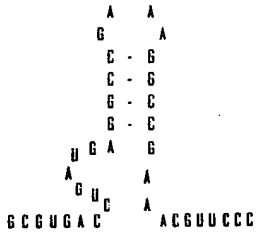
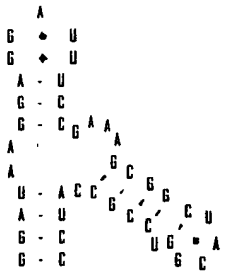


Figure 28: Erk modulated Nucleic Acid Sensor Molecule



HAMMERHEAD (HH)

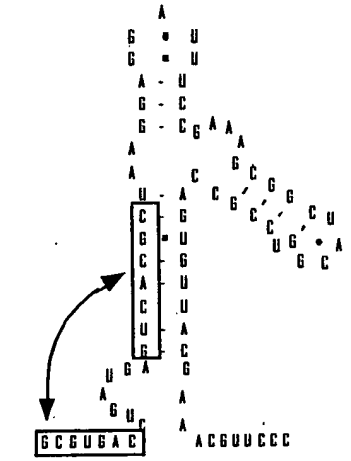
(SEQ ID NO. 40)



ERK SENSOR COMPONENT

K_D =300 pM

(SEQ ID NO. 41)



EHHI.3 sensor molecule

K_D =570 nM

(SEQ ID NO. 42)

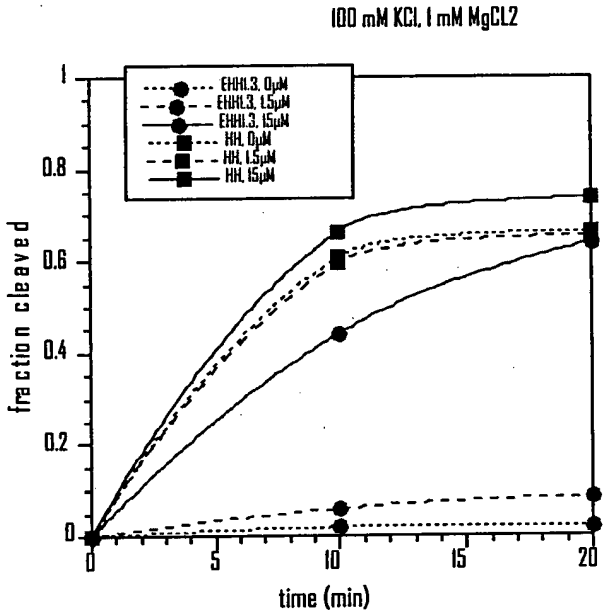


Figure 29: Half-Zinzyme Construct

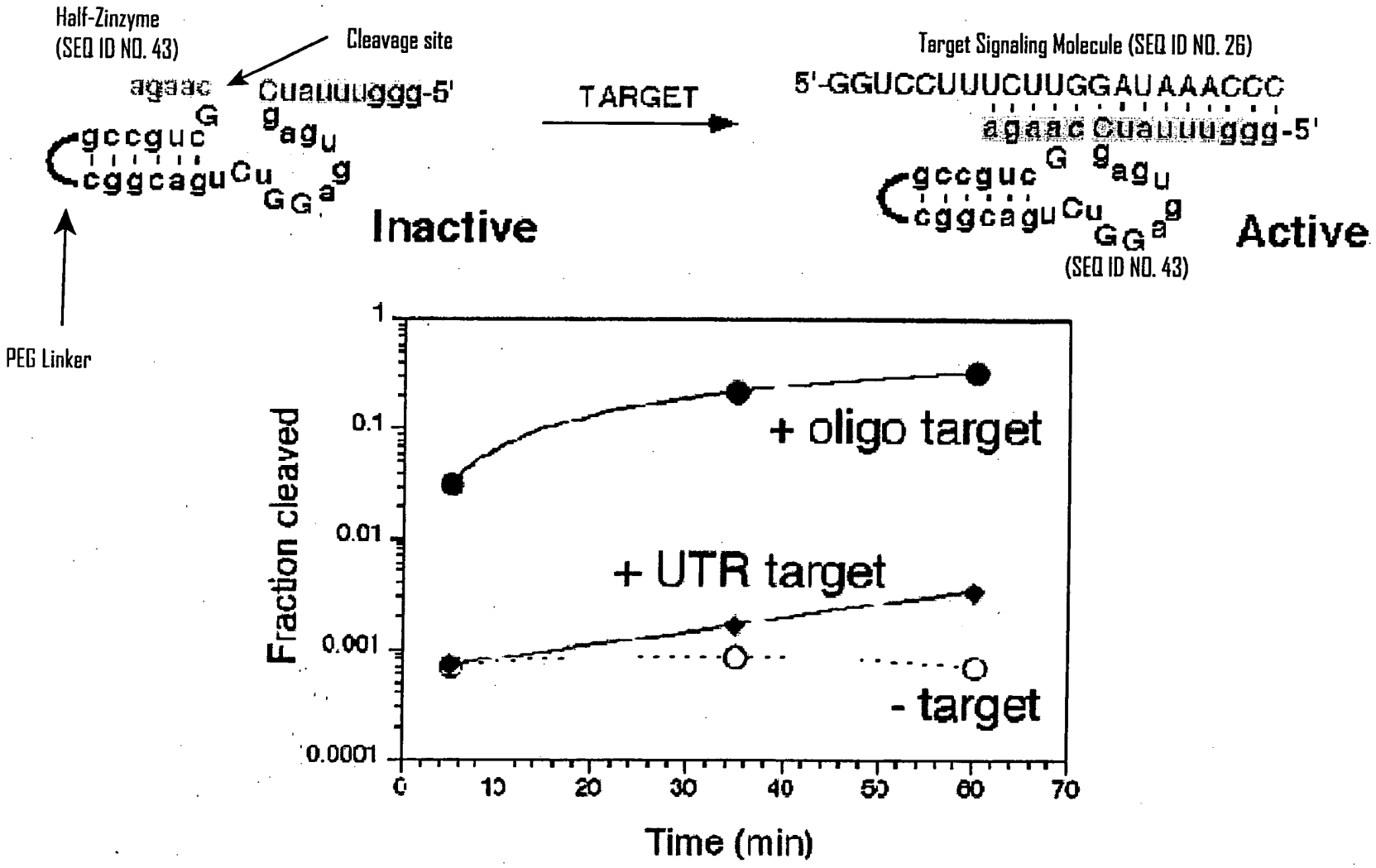
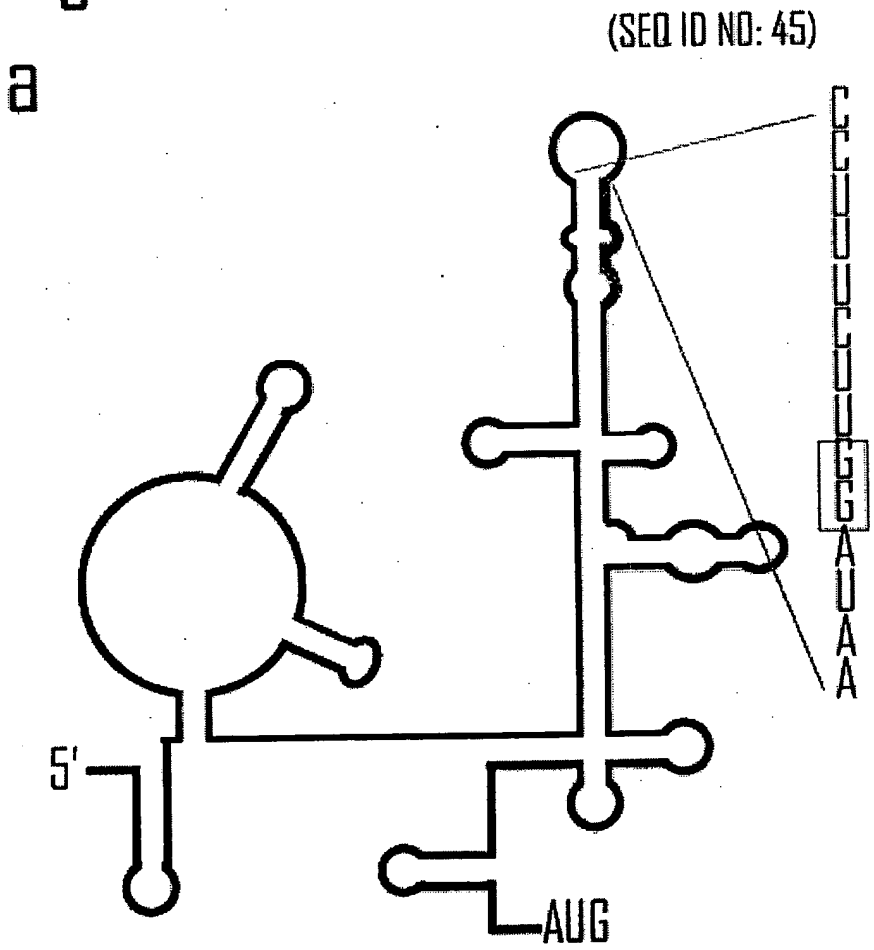


Figure 30


a
(SEQ ID NO: 45)
5'
AUG
AUAA

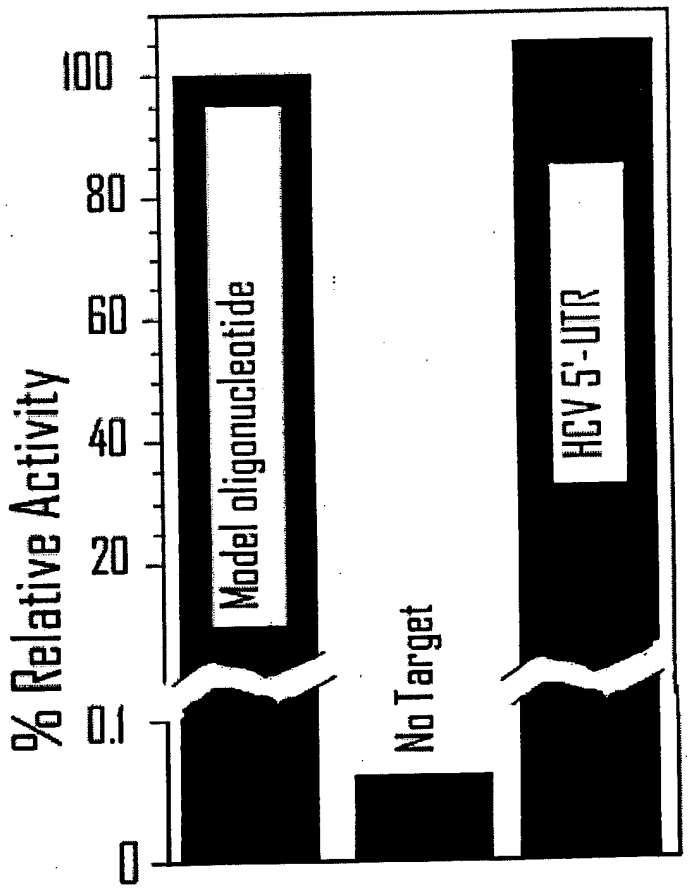

b
% Relative Activity
100
80
60
40
20
0.1
0
Model oligonucleotide
No Target
HCV 5'-UTR

Figure 31

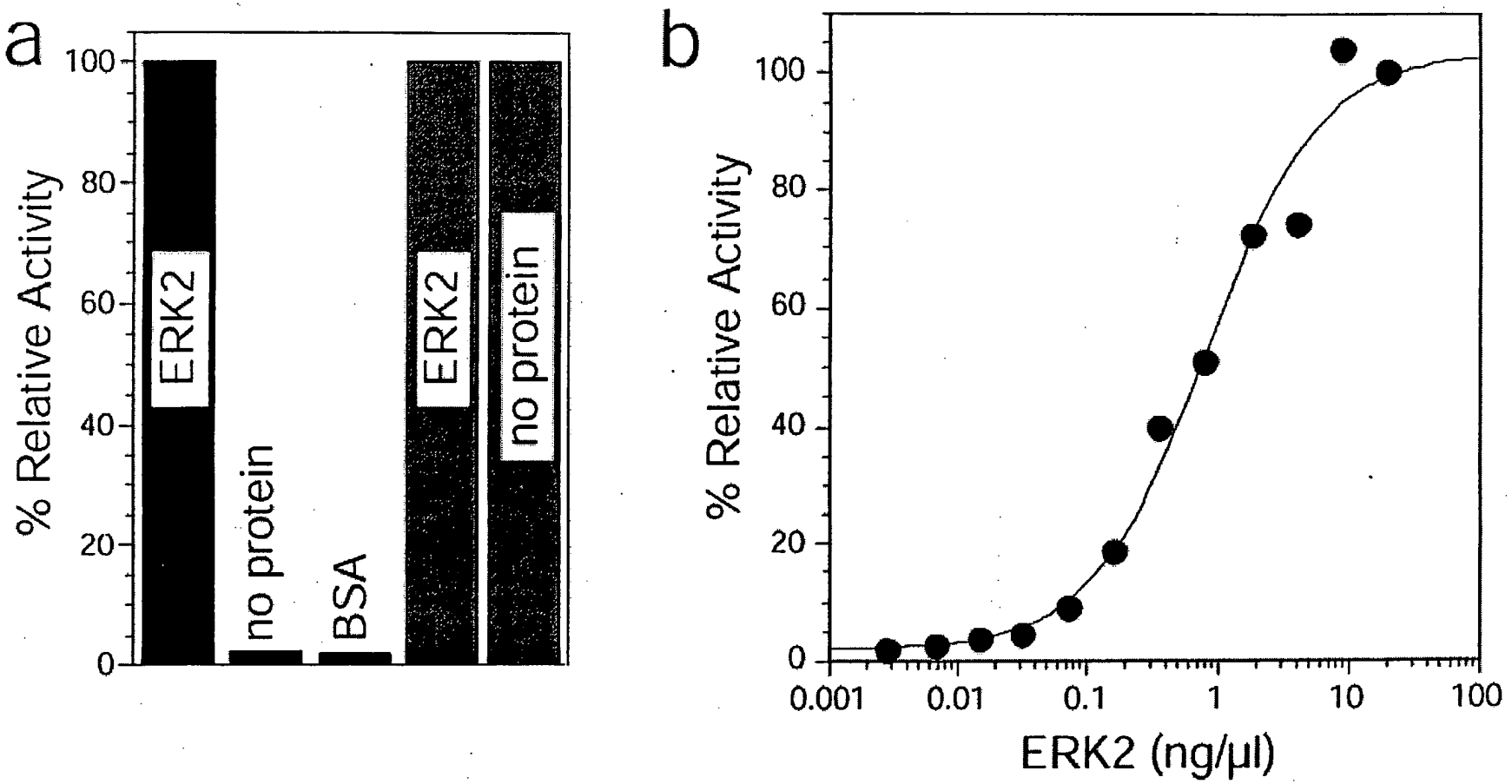

a
% Relative Activity
100
80
60
40
20
0
ERK2
no protein
BSA
ERK2
no protein
b
% Relative Activity
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
ERK2 (ng/µl)

Figure 32

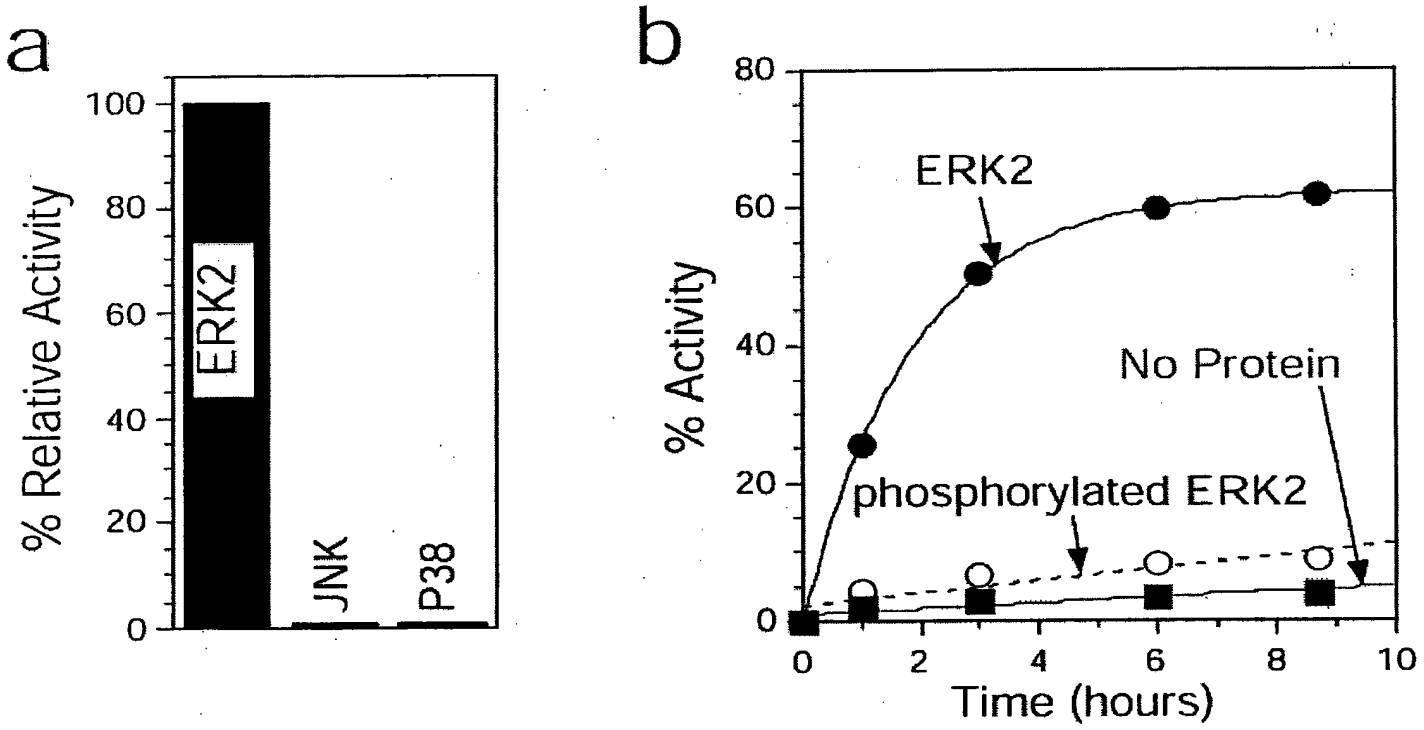


Figure 33: Halfzyme Ligase

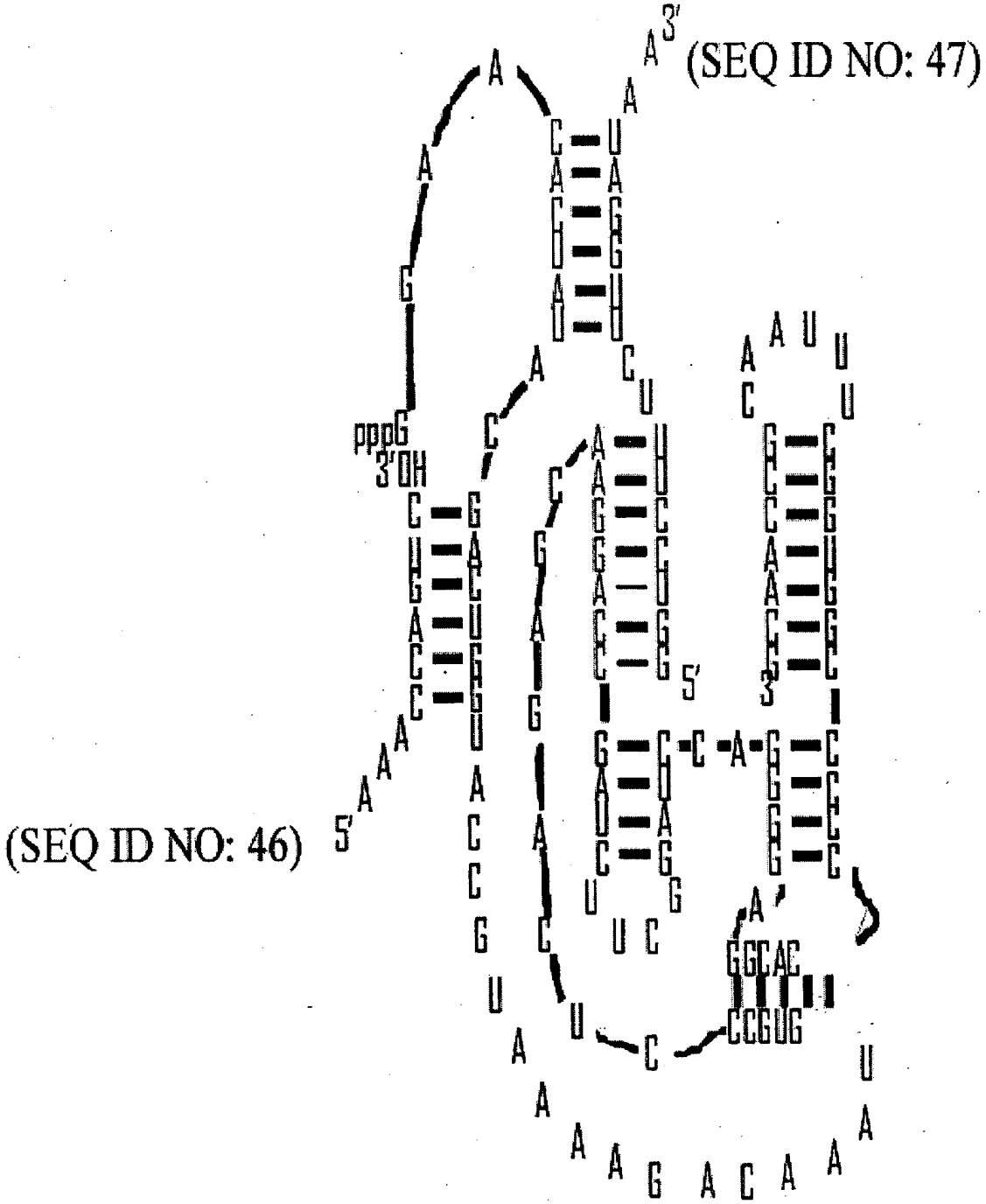
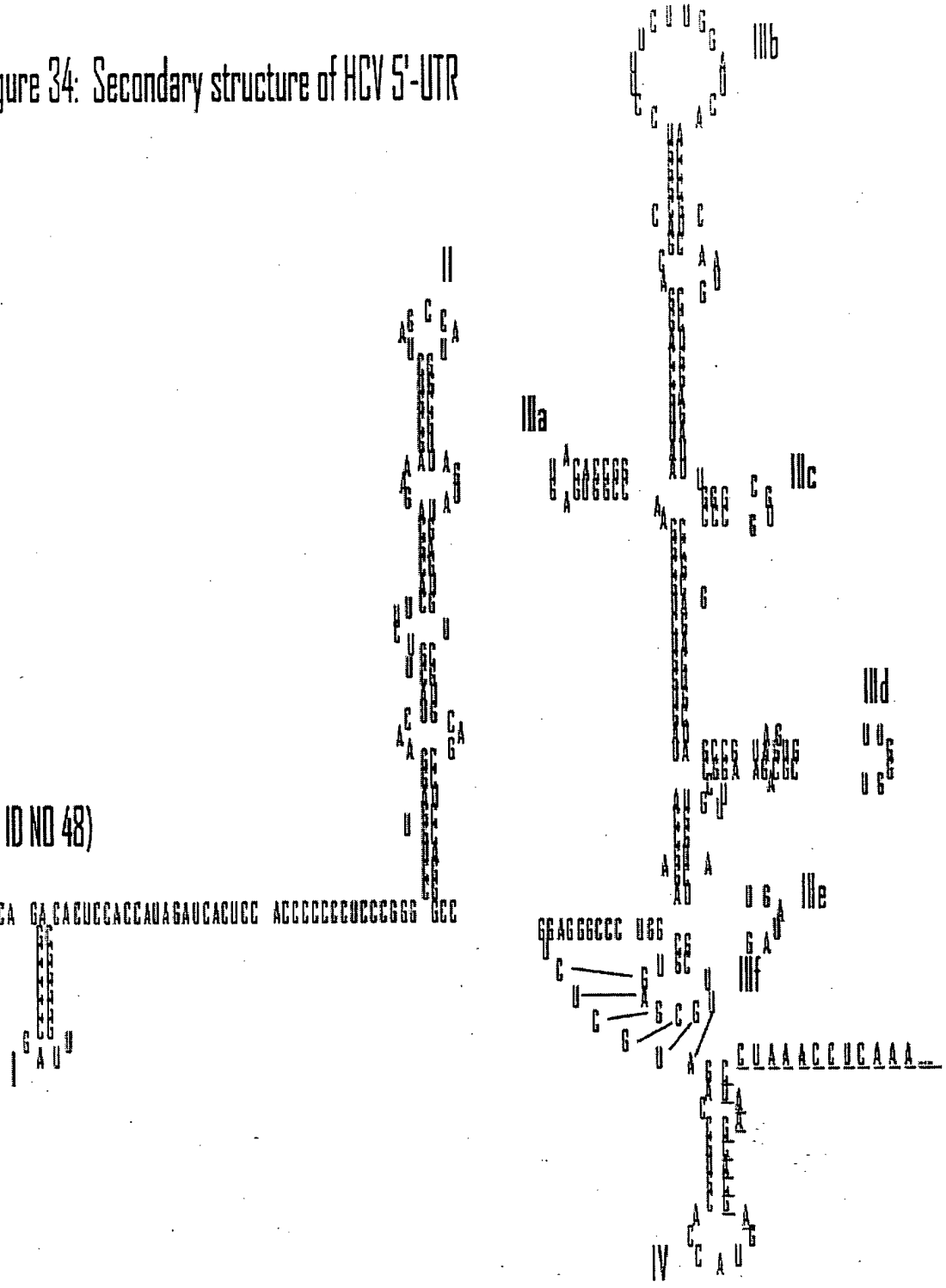


Figure 34: Secondary structure of HCV 5'-UTR



(SEQ ID NO 48)